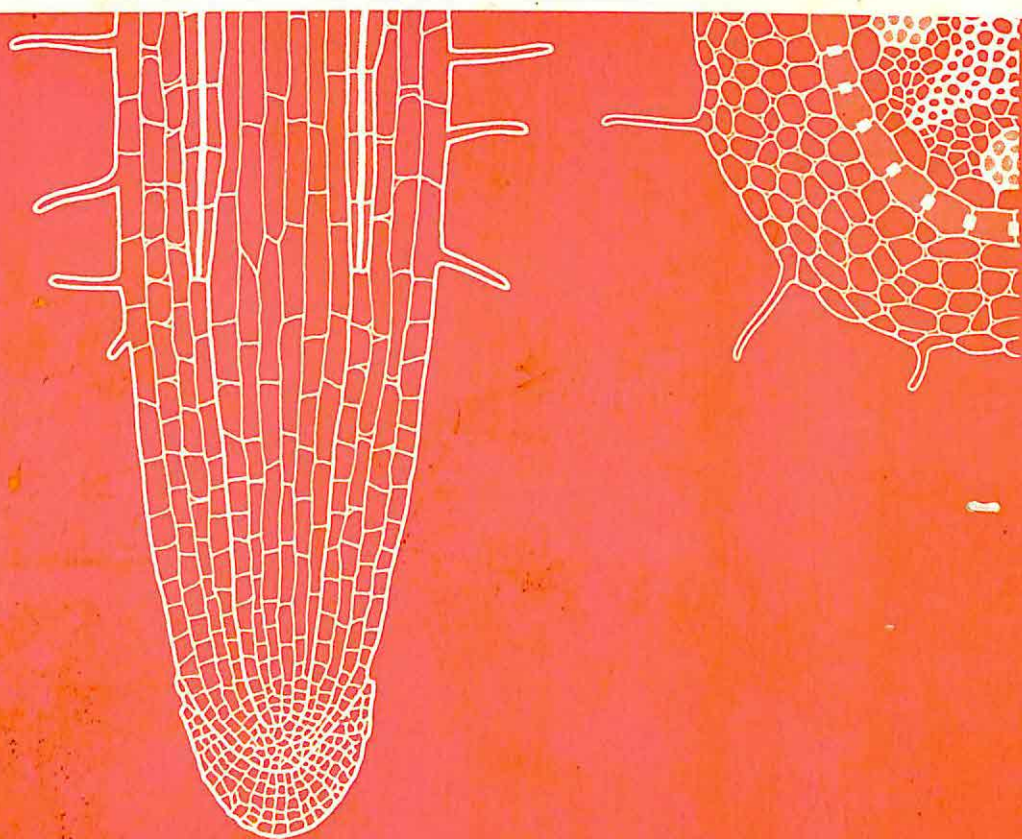


INTRODUCTION TO BIOPHYSICAL PLANT PHYSIOLOGY



PARK S. NOBEL

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INTRODUCTION TO
BIOPHYSICAL PLANT PHYSIOLOGY

A Series of Books in Biology

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INTRODUCTION TO
**BIOPHYSICAL
PLANT PHYSIOLOGY**

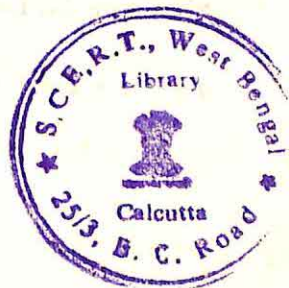
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9 8 7 6 5 4 3 2 1

CONTENTS

Preface xi

1 CELLS 1

Cell Structure 1

Generalized Plant Cell Leaf Cells Cells of Vascular Tissue Root Cells

Diffusion 9

Fick's First Law Continuity Equation and Fick's Second Law Time-Distance Relation for Diffusion.

Membrane Structure 19

Membrane Models Organelle Membranes

Membrane Permeability 26

Concentration Difference Across a Membrane Permeability Coefficient
Diffusion and Cellular Concentration

Cell Walls 32

Chemistry and Morphology Diffusion Across Cell Walls Stress-Strain
Relations of Cell Wall

Problems 41

References 42

2 WATER 44

Physical Properties 45

Hydrogen Bonding Surface Tension Capillary Rise Capillary Rise in the
Xylem Tensile Strength Electrical Properties

Chemical Potential 54

Free Energy and Chemical Potential Analysis of Chemical Potential Standard
State Hydrostatic Pressure Water Activity and Osmotic Pressure The Van't
Hoff Relation Matric Potential Water Potential

Central Vacuole and Chloroplasts 70

Water Relations of the Central Vacuole Boyle-Van't Hoff Relation Osmotic Responses of Chloroplasts

Water Potential and Plant Cells 77

Incipient Plasmolysis Activity and Water Potential of Water Vapor Plant-Air Interface Pressure in the Cell Wall Water Water Flux

Problems 88

References 89

3 SOLUTES 91

Chemical Potential of Ions 92

Electrical Potential Electroneutrality and Membrane Capacitance Activity Coefficients of Ions Nernst Potential Example of E_{N_R}

Fluxes and Diffusion Potentials 100

Flux and Mobility Diffusion Potential in a Solution Membrane Fluxes Membrane Diffusion Potential—Goldman Equation Application of the Goldman Equation Donnan Potential

Active Transport 118

Electrogenicity Q_{10} , a Temperature Coefficient Ussing-Teorell Equation Example of Active Transport Carriers, Solute Uptake, and the Michaelis-Menten Formalism

Principles of Irreversible Thermodynamics 138

Fluxes, Forces, and Onsager Coefficients Water and Solute Flow Volume Flow, L_p , and σ Values of Reflection Coefficients

Solute Movement Across Membranes 148

The Influence of Reflection Coefficients on Incipient Plasmolysis Extension of the Boyle-Van't Hoff Relation Reflection Coefficients of Chloroplasts

Problems 156

References 159

4 LIGHT 162

Wavelength and Energy 163

Light Waves Energy of Light Radiant Flux and Illumination Sunlight

Absorption of Light by Molecules 175

Role of Electrons in Absorption Event Electron Spin and State Multiplicity Molecular Orbitals Light Absorption by Chlorophyll

De-excitation 185

Fluorescence, Phosphorescence, and Radiationless Transitions Competing Pathways for De-excitation Lifetimes and Quantum Yields

Absorption Spectra and Action Spectra 192

Vibrational Sublevels in an Energy Level Diagram The Franck-Condon Principle Absorption Bands and Absorption Coefficients Conjugation Action Spectra Absorption and Action Spectra of Phytochrome

Problems 210

References 212

5 PHOTOSYNTHESIS 214

Chlorophyll—Chemistry and Spectra 217Types and Structures Absorption and Fluorescence Emission Spectra
Absorption Bands *in vivo**Other Photosynthetic Pigments* 224

Carotenoids Phycobilins

Excitation Transfers Among Photosynthetic Pigments 230Resonance Transfer of Excitation Transfers of Excitation Between Photosynthetic
Pigments Excitation Trapping*Photosynthetic Units and Enhancement Effects* 239

Photosynthetic Units Excitation Processing by Photosynthetic Units

Photosynthetic Action Spectra and Enhancement Effects Composition of the Two
Photosystems*Electron Flow and Photophosphorylation* 246

Electron Flow Components of the Electron Transfer Pathway Types of

Electron Flow Photophosphorylation

Problems 256*References* 258

6 BIOENERGETICS 260

Gibbs Free Energy 261Chemical Reactions and Equilibrium Constants Interconversion of Chemical and
Electrical Energy Redox Potentials*Biological Energy Currencies* 271

ATP, Structure and Reactions Gibbs Free Energy Change for ATP Formation

NADP-NADPH₂ Redox Couple*Chloroplast Bioenergetics* 279Redox Couples Coupling of ATP Formation to Electron Flow Coupling
Mechanism*Mitochondrial Bioenergetics* 288

Electron Flow Components—Redox Potentials Oxidative Phosphorylation

Energy Flow in the Biosphere 293Incident Light—Stefan-Boltzmann Law Absorbed Light and Photosynthetic
Efficiency Food Chains and Material Cycles*Problems* 298*References* 300

7 LEAVES 302

Resistances 303Resistance of Unstirred Boundary Layer Adjacent to Leaf Unstirred Layer
Thickness Stomata Stomatal Resistance Cuticular Resistance Resistance
of Intercellular Air Spaces Fick's First Law and Resistances*Water Vapor Fluxes Accompanying Transpiration* 313

Resistance Network Values of Resistances Leaf Water Vapor

Concentrations	Examples of Relative Humidity and c_{wv} in Leaves	Fluxes of Water Vapor
	Stomatal Control of J_{wv}	

CO₂ Fluxes Accompanying Photosynthesis 325

Resistance Network	Mesophyll Area	Cell Wall Resistance	Plasmalemma Resistance
Cytoplasmic Resistance	Mesophyll Resistance	Chloroplast Resistance	Photosynthesis
Respiration and Photorespiration	Comprehensive CO ₂ Resistance Network	Compensation Points	Fluxes of CO ₂
Relationships Between J_{wv} and J_{CO_2}			

Energy Fluxes 343

Solar Radiation	Absorbed Infrared Radiation	Emitted Infrared Radiation
Values for a , a_{IR} and e_{IR}	Net Radiation	Examples for Radiation Terms
Heat Conduction and Convection	Examples of Heat Conduction	Heat Flux
Accompanying Transpiration	Heat Flux for Dew or Frost Formation	
Examples of Frost and Dew Formation	Shaded Leaves Within Plant Communities	

Problems 362

References 364

8 PLANTS 366

Gaseous Fluxes Above the Leaf Canopy 367

Wind Velocity	Fluxes	Eddy Diffusion Coefficients	Resistance of Air
Above the Canopy	Transpiration and Photosynthesis	J_{CO_2} and $c_{CO_2}^{ta}$	
J_{wv} and c_{wv}^{ta}			

Gas Fluxes Within Plant Communities 374

Eddy Diffusion Coefficient and Resistance	Water Vapor	Illumination Values
of Foliar Absorption Coefficients	Light Compensation Point	CO ₂
Concentrations and Fluxes	CO ₂ at Night	

Soil 382

Thermal Properties	Soil Energy Balance	Soil Water Potential	Darcy's Law
Soil Hydraulic Conductivity Coefficient	Fluxes for Cylindrical Symmetry		

Water Movement in the Xylem and Phloem 390

Root Tissues	The Xylem	Poiseuille's Law	Applications of Poiseuille's Law
The Phloem	Phloem Contents and Velocity of Movement	Mechanism of Phloem Flow	Possible Values for Components of the Water Potential in the Phloem

The Soil-Plant-Atmosphere Continuum 401

Values of Water Potential Components	Resistances and Areas	Diurnal Changes
--------------------------------------	-----------------------	-----------------

Problems 408

References 410

APPENDICES 413

I *Frequently Used Variables and Their Units* 415

II *Frequently Used Constants and Their Numerical Values* 419

III *Conversion Factors* 422

IV	<i>Abbreviations and Symbols</i>	427
V	<i>Natural and Common Logarithms</i>	431
VI	<i>Calculus</i>	435
VII	<i>Gibbs Free Energy and Chemical Potential</i>	451
VIII	<i>Further Comments on Irreversible Thermodynamics</i>	461
IX	<i>Measurement of Electrical Potential Differences Across Biological Membranes</i>	466

ANSWERS TO PROBLEMS	473
---------------------	-----

INDEX	481
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PREFACE

Traditionally, plant physiology is divided into such areas as water relations, mineral nutrition, metabolism, photosynthesis, and the enormous area of growth and development. Many of these areas overlap with cell physiology, where the differences between plants and animals tend to break down. One goal of a plant or cell physiologist is to understand such fields in formal physical and chemical terms. Accurate models can then be constructed and the response to the environment predicted. Such physicochemical explanations are so far available for only certain areas of plant and cell physiology, as for example, ion and water movements across cellular membranes, the photosynthetic conversion of radiant energy from the sun into other forms of energy, and gaseous fluxes for leaves. These are some of the topics discussed in this book.

Chapters 1-3 present the physical description of water relations and ion transport involving plant cells. In Chapter 1, after discussing the concept of diffusion, we will consider the physical barriers to free diffusion caused by cellular and organelle membranes. The other physical barrier associated with plant cells is the massive cell wall, which limits the size and consequently the contents of the cells. In Chapter 2, in the treatment of the movement of water through cells in response to physical forces, we will make use of the thermodynamic argument of chemical potential gradients. Chapter 3 considers the topic of solute movement into and out of plant cells, leading to an explanation of electrical potential differences across membranes. Formal criteria for distinguishing diffusion from active transport will also be developed, and an important parameter called the reflection coefficient will be derived, using concepts from irreversible thermodynamics. The use of reflection coefficients permits an evaluation of osmotic pressure effects across membranes under real, rather than idealized, conditions. The thermo-

dynamic arguments used to describe ion and water movements are equally applicable to animal cells.

The second part of the book deals with the interconversion of various forms of energy. In Chapter 4 we consider the properties of light and its absorption. Following light absorption by nonliving material, the radiant energy is usually rapidly converted to heat. The arrangement of photosynthetic pigments and their special molecular structures allows some radiant energy from the sun to be converted by plants into other forms of energy before the eventual degradation of such light energy to thermal energy. In Chapter 5 we discuss the particular features of chlorophyll and the accessory pigments for photosynthesis which allow this energy conversion. Light energy absorbed by chloroplasts leads to the formation of ATP and NADPH₂. These compounds represent currencies for carrying chemical and electrical (redox potential) energy, respectively. How much energy they actually carry is discussed in Chapter 6. In the last two chapters we will consider the various forms in which energy enters and leaves the plant, i.e., the relationship between the environment, energy fluxes, and the plant. Moreover, the numerous resistances affecting the movement of both water vapor accompanying transpiration and carbon dioxide during photosynthesis will be discussed in detail. Since these and other topics depend on material introduced elsewhere in the book, the text is extensively cross-referenced so that particular aspects can be selected for consideration.

This text has evolved from *Plant Cell Physiology: A Physicochemical Approach* (W. H. Freeman and Company, San Francisco, 1970). Chapters on environmental interactions of the leaf (Ch. 7) and the whole plant (Ch. 8) have been added, and the treatment of other biophysical aspects has been greatly expanded (including new appendices). Numerous other changes have emanated from the best laboratory for such a book, the classroom. The main emphasis is on concepts that are important for a broad biological understanding of processes, particularly *in vivo*. Physiological consequences of physical principles are pointed out, often by means of calculations. Although some knowledge of elementary calculus and physics is useful, the intent is to encourage rigorous development in the student without placing undue demands on his background or memory. The inspiration for this approach to the study of plants came from Professor Jack Dainty. Dr. Steve Cheung critically examined the present manuscript. The author also gratefully acknowledges the comments of Professor Robert S. Loomis and Professor Douglas C. Spanner, whose thorough reviews substantially improved the book, as well as various writers and publishers who granted permission to use certain figures.

P.S.N.

Ye rigid Ploughmen! Bear in mind
Your labour is for future hours.
Advance! Spare not! Nor look behind!
Plough deep and straight with all your powers!

from *The Plough*, R. H. Horne, 1848.

INTRODUCTION TO
BIOPHYSICAL PLANT PHYSIOLOGY

CELLS

Cell structure

Before embarking on a formal consideration of diffusion and other biophysical topics, we will outline the structure of certain plant cells and tissues. This will serve to introduce many of the anatomical terms needed throughout the rest of the book.

Generalized plant cell. Figure 1.1 depicts a representative leaf cell from a higher plant and illustrates the larger subcellular structures found in a plant cell. Surrounding the protoplast is a wall composed mainly of cellulose and other polysaccharides; these polymers help provide rigidity to individual cells as well as to the whole plant. Since the cell wall contains numerous relatively large interstices, it does not serve as the main permeability barrier to water or solute movement into plant cells. Inside the cell wall and surrounding the cytoplasm is a cell membrane known as the plasma membrane or *plasmalemma*. The permeability of this membrane depends on the particular solute and so the plasmalemma regulates what enters and leaves a plant cell. The cytoplasm contains organelles like chloroplasts and mitochondria, distinct membrane-surrounded compartments in which energy can be converted from one form to another. Also in the cytoplasm are the nucleus, special microbodies such as "peroxisomes," numerous ribosomes, proteins, as well as many other macromolecules and structures which influence the thermodynamic properties of the water there.

In mature cells of higher plants as well as a number of lower (less advanced) plants, there is a large central aqueous phase referred to as the *vacuole*. This vacuole is surrounded by a membrane known as the *tonoplast*. The tonoplast is usually quite large in area since the central vacuole can occupy up to 90% of the volume of a mature cell. Small cytoplasmic vesicles

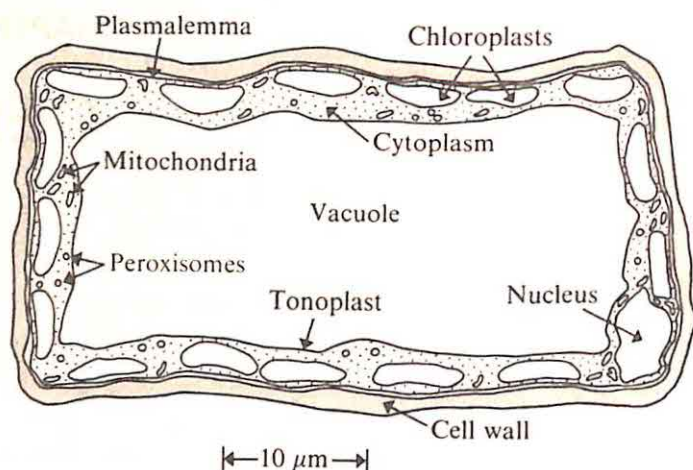


Figure 1.1

Schematic representation of a mature mesophyll cell from the leaf of a higher plant, suggesting some of the complexity resulting from the presence of many membrane-surrounded subcellular bodies.

are often found associated with the tonoplast, and these may be involved in transport. The relatively simple aqueous solution in the central vacuole contains mainly inorganic ions or organic acids as solutes, although considerable amounts of sugars and amino acids may be present in some species. Water uptake into this vacuole occurs during cell growth.

One immediate impression of plant cells is the great prevalence of membranes. In addition to surrounding the protoplasm, membranes also separate various compartments from the cytoplasm. Diffusion of substances across these membranes is much more difficult than diffusion within the compartments. Thus organelle and vacuolar membranes can effectively control the contents and consequently the reactions occurring in the particular compartments which they surround. Diffusion can also impose limitations on the overall size of a cell, since the time for diffusion increases with the square of the distance, as we will quantitatively consider in the next section.

Although many plant cells share most of the features indicated in Figure 1.1, they are remarkably diverse in size. The nearly spherical cells of the green alga *Chlorella* are approximately 4×10^{-6} meter ($4 \mu\text{m}$) in diameter (see App. IV for a complete list of abbreviations). On the other hand, some species of the intertidal green alga *Valonia* have multinucleated cells as large as a few centimeters in diameter. The genera *Chara* and *Nitella* include fresh and brackish water green algae having large internodal cells (see Fig. 3.4) which may be 10 cm long and 1 mm in diameter. Such giant algal cells have

proved extremely useful for studying ion fluxes, as we will consider in Chapter 3.

Leaf cells. A transverse section of a leaf as diagrammed in Figure 1.2 further illustrates some of the heterogeneity of cell types encountered in plants. It also serves to introduce certain anatomical features important for photosynthesis and transpiration. Leaves of plants like pea and spinach are generally 4 to 10 cells thick, which corresponds to a few hundred microns (Fig. 1.2). An *epidermis* occurs on both the upper and the lower sides of a leaf and is usually one layer of cells thick. The cytoplasm of epidermal cells generally appears colorless since very few if any chloroplasts are present there (depending on the plant species). Epidermal cells have a waterproof *cuticle* on the atmospheric side (Fig. 1.2). The cuticle contains cutin, a diverse group of complex polymers composed principally of esters of 16- and 18-carbon monocarboxylic acids that have two or three hydroxyl groups. Since cutin is relatively inert and also resists enzymatic degradation by microorganisms, it is often well preserved in fossil material. We are mainly concerned with its role in preventing water loss from a leaf.

Between the two epidermal layers is the leaf *mesophyll*, which is composed

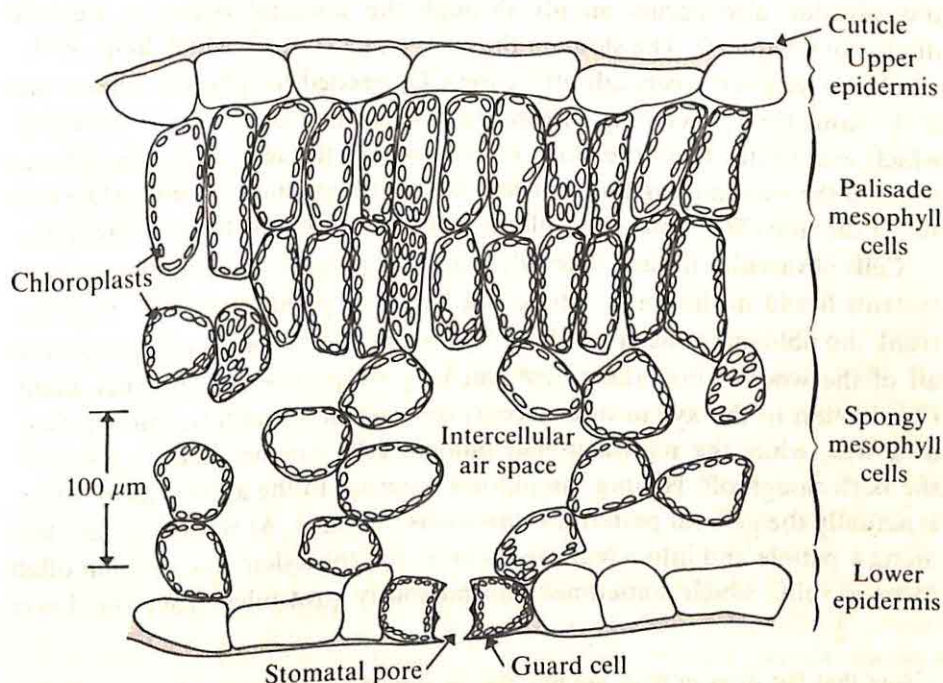


Figure 1.2

Schematic transverse section through a leaf, indicating the arrangement of various cell types.

of chloroplast-containing “palisade” and “spongy” cells. The palisade cells are usually elongated at right angles to the upper epidermis and are found immediately beneath it (Fig. 1.2). The spongy mesophyll cells, between the lower epidermis and the palisade mesophyll cells, are loosely packed with conspicuous intercellular air spaces. In fact, most of the surface area of both spongy and palisade mesophyll cells is exposed to the air in the intercellular spaces. A spongy mesophyll cell from a pea leaf is about $40 \times 40 \times 40 \mu\text{m}$ and contains approximately 40 chloroplasts. (As Fig. 1.2 illustrates, the cell is by no means geometrically regular in shape, so that dimensions here indicate only an overall size.) A neighboring palisade cell is generally more oblong and can be $30 \times 30 \times 80 \mu\text{m}$, containing slightly over 50 chloroplasts. In most pea leaves about 70% of the chloroplasts are in the palisade cells, which generally outnumber the spongy mesophyll cells nearly 2 to 1.

The pathway of least resistance for gases to cross an epidermis—and thus to enter or to exit from a leaf—is through the adjustable space between a pair of guard cells (Fig. 1.2). This pore is called a *stoma* or *stomate* (plural: stomata and stomates, respectively) and it can open and close. The stomatal pores allow for the entry into the leaf of CO_2 and for the release of the photosynthetically produced O_2 . The inevitable loss of water vapor by transpiration also occurs mainly through the stomatal pores, as we will discuss in Chapter 7. The stomata thus serve as a control, which helps strike a balance between freely admitting the CO_2 needed for photosynthesis and at the same time preventing excessive loss from the plant of the water vapor which evaporates from the walls of cells within the leaf and then diffuses out into the surrounding atmosphere. Air pollutants such as ozone (O_3) and sulfur dioxide (SO_2) also enter plants primarily through the open stomates.

Cells of vascular tissue. The xylem and the phloem make up the vascular systems found in the roots, stems, and leaves of plants (Fig. 1.3). In a tree trunk the phloem is one layer of the bark while the xylem constitutes almost all of the wood. Thus, the xylem can help support a tree or other plant. Conduction in the xylem often occurs only in the outermost annual ring* of a tree, while the nonfunctional phloem cells can be shed as pieces of the bark slough off. Having the phloem external to the xylem, as in a tree, is actually the general pattern for the stems of plants. As we follow the stem along a petiole and into a leaf, we observe that the xylem and phloem often form a vein, which sometimes conspicuously protrudes from the lower

* Note that the rings in trees are not always annual. In many desert species a ring forms when large and, later, small xylem cells are produced following a suitable rainy period, and this can occur more than once or sometimes not at all in a given year.

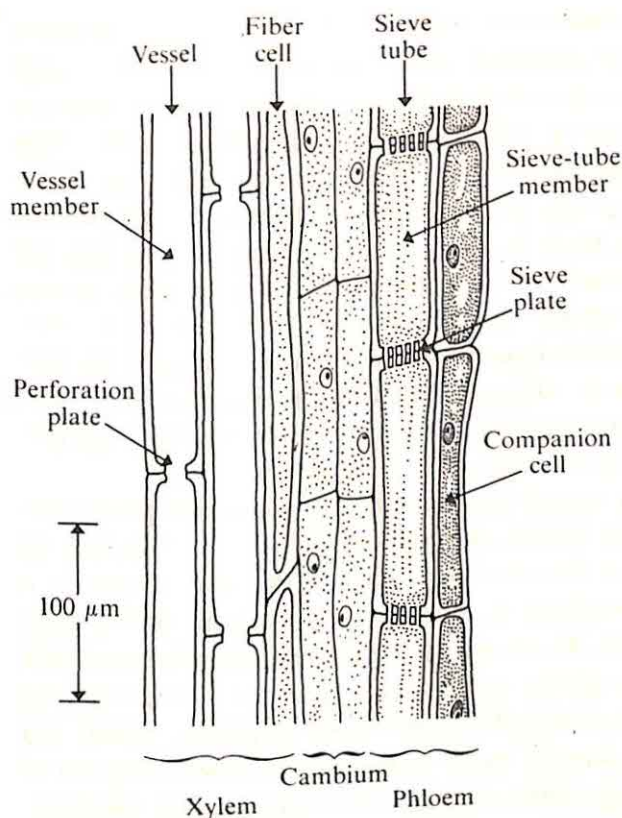


Figure 1.3

Longitudinal section through part of a vascular bundle in a stem, showing elements of both the xylem and the phloem.

surface of the leaf. Reflecting the orientation originating in the stem or the trunk, the phloem is found beneath the xylem in the vascular tissue of a leaf, i.e., the phloem is located on the side of the lower epidermis. The vascular system branches and rebranches as it crosses a dicotyledonous leaf, becoming smaller (in cross-section) at each step. (Monocotyledons characteristically have parallel-veined leaves.) Individual mesophyll cells in the leaf are never very distant from the vascular tissue, being separated from the xylem or phloem by no more than a few cells.

The upward movement of water and nutrients from the soil to the upper portions of a plant occurs primarily in the xylem. The xylem sap generally contains about 10 mM inorganic nutrients plus organic forms of nitrogen that are metabolically produced in the root. The xylem is a tissue of various cell types which we will consider in more detail in the last chapter (p. 391) when water movement in plants is briefly discussed in terms of the water

potential. The conducting cells in the xylem are the *tracheids* and the *vessel members*, both of which are arranged end-to-end in long linear files. Contiguous vessel members are joined at *perforation plates*, thereby forming a continuous tube or vessel (cf. Fig. 1.3). The conducting cells having lost their protoplasts and, in the case of vessel members, much of their end walls, the remaining cell walls border a low resistance channel for the passage of solutions. Water moves from the root, up along the stem, through the petiole and then to the leaves in these hollow xylem cells, with motion occurring in the direction of decreasing hydrostatic pressure (see pp. 393–395). Ions and other solutes accompany such movement of water to the leaf. Some solutes leave the xylem along the stem on the way to a leaf, while others diffuse or are actively transported across the plasmalemmas of various leaf cells adjacent to the conducting cells of the xylem.

The movement of most *organic* compounds throughout the plant takes place in the other vascular system, the phloem. A portion of the photosynthetic products made in the mesophyll cells of the leaf diffuses or is actively transported across cellular membranes until it reaches the conducting cells of the leaf phloem. By means of the phloem, the photosynthetic products—which are then mainly in the form of sucrose—are distributed throughout the plant. The carbohydrates produced by photosynthesis and certain other substances generally move in the phloem toward regions of lower concentration, although diffusion is not the mechanism for the movement, as we will indicate later (see pp. 397, 401). The phloem is a tissue consisting of several types of cells. In contrast with the xylem, however, the conducting cells of the phloem contain cytoplasm (see Fig. 1.3 and the discussion in Ch. 8). The conducting cells in the phloem are called *sieve cells* and *sieve-tube members*. They are joined end-to-end and thus form a transport system throughout the plant. Although these phloem cells often contain no nuclei at maturity, they remain metabolically active.

Root cells. Roots serve to anchor plants in the ground as well as to absorb water and nutrients from the soil and then to conduct these substances upward to the stem. In order to help understand uptake into the plant, we will examine the functional zones that occur along the length of a root (see Epstein).

At the extreme tip of a root is the root cap (Fig. 1.4a). It consists of relatively undifferentiated cells, frequently containing starch, which become scraped off as the root grows into new regions of the soil. Cell walls in the root cap often are mucilaginous in consistency, which might serve to reduce friction with soil particles. Behind the root cap is a meristematic region where the cells rapidly divide. Cells in this *apical meristem* tend to be isodiametric

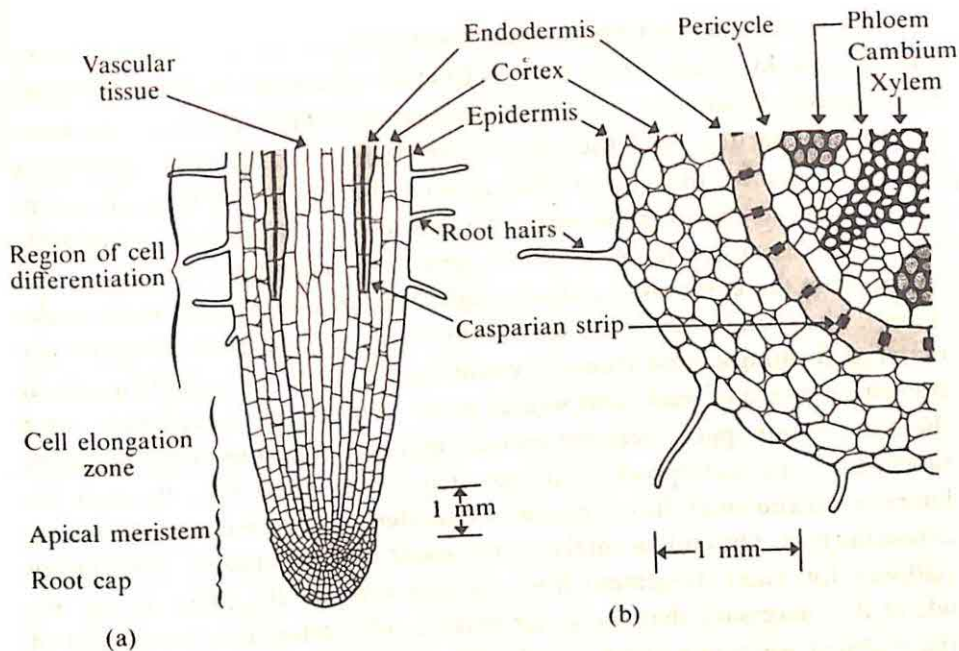


Figure 1.4

Schematic diagrams of a root: (a) longitudinal section, indicating the zones which can occur near the root tip (b) cross-sectional view approximately 1 cm back from the tip, indicating the arrangement of the various cell types.

and have thin cell walls. Next to this is a region of *cell elongation* in the direction of the root axis. Such elongation mechanically pushes the root tip through the soil, causing cells of the root cap to slough off by abrasion with soil particles. Sometimes the region of dividing cells is not spatially distinct from the elongation zone. Also, cell size and the extent of the zones vary with both plant species and physiological status (see Esau for details).

The next region indicated in Figure 1.4a is that of *cell differentiation*, where the cells begin to assume more highly specialized functions. The cell walls become thicker and elongation is greatly diminished. The epidermal cells develop fine projections, radially outward from the root, called *root hairs*. These root hairs greatly increase the surface area across which water and nutrients can enter a plant. In fact, most of the water usually enters the root system in the region where the root hairs are located (generally a few millimeters or centimeters from the tip). As we follow the root upward, its surface becomes less permeable to water and its interior more involved with conducting water upward to the stem. Since water movement into the root is discussed in Chapter 8, the discussion here will be restricted to some morphological features.

The region of the root where water absorption occurs has little or no waxy cuticle, and this is consistent with its function of taking up water from the soil. Figure 1.4b shows a cross-section of a root at the level where root hairs are found. Starting from the outside, we observe first the root epidermis and then a number of layers of cells known as the *cortex*. There are abundant intercellular air spaces in the cortex, facilitating the diffusion of O_2 and CO_2 within this tissue (such air spaces are generally lacking in vascular tissue). Inside from the cortex is a unique, single layer of cells known as the *endodermis*. The radial walls of the endodermal cells are impregnated with waxy material, forming a band around the cells known as the *casparian strip*, which prevents passage of water and solutes across that part of the cell wall. Since there are no air spaces between endodermal cells, and the radial walls are blocked by the waterproof casparian strip, water must pass through the lateral walls and enter the cytoplasm of endodermal cells in order to continue across the root. The endodermal cells represent the only instance in the entire pathway for water movement from the soil, through the plant, to the air, where it is necessary that the water enter a cell's cytoplasm. In the rest of the pathway water can move in cell walls or in the hollow lumens of xylem vessels.

Inside from the endodermis is a layer called the *pericycle*, which is typically one cell thick in angiosperms. The cells of the pericycle can divide and form a meristematic region, which in turn can lead to lateral or branch roots in the region just above the root hairs. Inside from the pericycle is the vascular tissue. The phloem generally occurs in two to eight or more strands located around the root axis (four strands, or a *tetrarch* arrangement, is illustrated in Fig. 1.4b). The xylem often occurs along the root axis with sections radiating out between the phloem strands. Water does not therefore have to cross the phloem in order to reach the root xylem. The tissue between the xylem and the phloem is the *cambium*, whose cells, upon division and differentiation, lead to xylem on the inside or phloem on the outside.

The endodermis surrounds the pericycle and vascular tissue for only a short length of the root. It does not begin until after the zone of cell elongation (Fig. 1.4a) and until a few phloem cells have differentiated. It breaks up just above the region of root hairs, where the root begins to widen appreciably as secondary xylem and secondary phloem are formed by the radial division of cambial layer cells. Thus, the main region for water entry into most roots is the zone of primary vascular tissue where there is an intact sleeve of endodermal cells. Toward the stem from this region the vascular tissue becomes larger in cross-section, the xylem is more devoted to conduction of water upward, and the phloem is needed to supply all the branch roots which have by then come together.

Our rather elementary discussion of leaves, vascular tissues, and roots leads to the following oversimplified but useful picture. The roots take up water from the soil along with nutrients required for growth. These are then conducted in the xylem to the leaves. Leaves of a photosynthesizing plant lose the water to the atmosphere along with a release of O_2 and an uptake of CO_2 . Carbon from the latter ends up in photosynthetate translocated in the phloem back to the root. Thus the xylem and the phloem serve as the "plumbing" which connects the two types of plant organs that functionally interact with the environment. To understand the details of such physiological processes we must turn to fields like calculus, thermodynamics, and photochemistry for the analytical methods. Our next step is to bring the abstract ideas of these fields into the realm of cells and plants, which means we need to make calculations using appropriate assumptions and approximations.

We will begin by describing diffusion (Ch. 1). In order to discuss water (Ch. 2) and solutes (Ch. 3), we will introduce the thermodynamic concept of chemical potential. This leads to a quantitative description of fluxes, electrical potentials across membranes, and the energy requirements for active transport of solutes. The energy conversion processes take place in the organelles. Specifically, light energy is absorbed (Ch. 4) by photosynthetic pigments located in the internal membranes of chloroplasts (Ch. 5) and then converted into other forms of energy useful to a plant (Ch. 6) or dissipated as heat (Ch. 7). The leaf (Ch. 7) as well as groups of plants (Ch. 8) also interact with the environment by means of exchanges of water and CO_2 . In our problem-solving approach to these topics we will pay particular attention to dimensions and ranges for the parameters as well as to the insights which can be gained only by developing the relevant formulae and then making calculations.

Diffusion

Diffusion is a spontaneous process leading to the net movement of a substance from some region to adjacent ones where that species has a lower concentration. It takes place in both the liquid and the gas phases associated with plants. Diffusion results from the random thermal motions of the molecules either of the solute and the solvent in the case of a solution, or of a gas in the case of air. The net movement caused by diffusion is a statistical phenomenon: there is a greater probability of molecules moving from the concentrated to the dilute region than vice versa. In other words,

more molecules per unit volume are present in the concentrated region than in the dilute one, and thus, more are available for diffusing toward the dilute region than are available for movement in the opposite direction. If isolated from external influences, diffusion of a neutral species will tend to even out any macroscopic concentration differences that are originally present in adjoining regions of a liquid or a gas. In fact, the randomizing tendency of such molecular Brownian movement is a good example of the increase in entropy, or decrease in order, that accompanies all spontaneous processes.

Diffusion is the mechanism for most, if not all steps by which CO_2 from the air reaches the sites of photosynthesis in chloroplasts. For instance, CO_2 diffuses from the atmosphere up to the leaf surface and then diffuses through the stomatal pores. After entering the leaf, diffusion of CO_2 occurs within intercellular air spaces, such as those indicated in Figure 1.2. Next, CO_2 diffuses across the wet cell wall, crosses the plasmalemma of a leaf mesophyll cell, and then diffuses through the cytoplasm to reach the chloroplasts. Finally, CO_2 enters a chloroplast and diffuses up to the enzymes that are involved in carbohydrate formation. If the enzymes fixed all the CO_2 in their vicinity, and no other CO_2 were to *diffuse* in from the atmosphere surrounding the plant, photosynthetic processes would stop.* Diffusion is likewise involved in many other aspects of plant physiology—here we will pay particular attention to the diffusion of substances across membranes. The mathematical formulation necessary for understanding both diffusion across a membrane and diffusion in free solution will be developed in this chapter.

Fick's first law. Fick in 1855 was one of the first to examine diffusion on a quantitative basis. For such an analysis we need to consider the concentration (c_j) of some species j in a solution. (The subscript j simply calls attention to the fact that we are considering only one species out of the many that could be present.) We will assume that the concentration of species j in some region is less than in a neighboring one. A net migration of molecules then occurs by diffusion from the concentrated to the dilute region. Such a molecular flow down a concentration gradient is analogous to the flow of heat down a temperature gradient from a warmer region to a cooler one. The analogy is actually rather good (especially for gases), since both processes depend on the random thermal motion of the molecules. In fact, the differential equations and their solutions which are used to describe

* Note that in solution " CO_2 " can occur in the form of bicarbonate and also that the crossing of membranes does not have to be by diffusion, refinements that we will return to on pp. 329–333.

diffusion are those which had previously been developed to describe heat flow. (See Crank for a discussion of the mathematics of such general transport relations.)

To express diffusion quantitatively, we will consider a diffusive flux or flow of species j , J_j . We will restrict our attention to diffusion involving planar fronts of uniform concentration. (Fortunately, as we shall see, this has widespread application to situations of interest in biology.) We will let J_j be the amount of some species j crossing a certain area per unit time, e.g., moles of particles per cm^2 in a second. From reasoning based on an analogy with heat flow, Fick deduced that the "force," or causative agent, leading to the net molecular movement depends on the concentration gradient. A gradient is simply a measure of how a certain parameter changes with distance, e.g., the gradient in concentration of species j in the x -direction is $\partial c_j / \partial x$. (See p. 438 for the definition and properties of a partial derivative.) In general, *the flux of some substance is proportional to the appropriate force*, a relation which we will use repeatedly in this text. In the present instance the driving force is the negative of the concentration gradient of species j , which we will represent by $-\partial c_j / \partial x$ for diffusion in one dimension. (To appreciate why a minus sign occurs, we should recall that the direction of net diffusion is toward regions of lower concentration.) We can now write down the following relation showing the dependence of the flux on the driving force:

$$J_j = -D_j \frac{\partial c_j}{\partial x} \quad (1.1)$$

Equation 1.1 is commonly known as Fick's first law of diffusion, where D_j is the *diffusion coefficient* of species j . The partial derivative (discussed in App. VI) is used in Equation 1.1 to indicate the change in concentration in the x -direction of Cartesian coordinates at some moment in time (constant t) and for specified values of y and z . (For the cases that we will consider, the flux in the x -direction actually has the same magnitude at any value of y and z .)

The diffusion coefficient is often expressed in cm^2/sec . For instance, for c_j in moles/ cm^3 (hence, $\partial c_j / \partial x$ in moles/ cm^4) and J_j in moles/ $\text{cm}^2\text{-sec}$, D_j is in cm^2/sec .* Since D_j varies with concentration and temperature, it is properly called a *coefficient* in the general case. In certain applications,

* We will generally write the unit of flux as moles/ $\text{cm}^2\text{-sec}$; for less common quantities we will also use the equivalent and less ambiguous forms, moles/($\text{cm}^2\text{-sec}$) or moles $\text{cm}^{-2} \text{sec}^{-1}$.

however, we can obtain sufficient accuracy by treating D_j as if it were a constant. By convention, a flux in the direction of increasing x is called positive (from left to right in Fig. 1.5). Since a net flow occurs toward regions of lower concentration, the minus sign is indeed needed in Equation 1.1. Fick's first law—which has been amply demonstrated experimentally—is the starting point for our discussion of diffusion.

Continuity equation and Fick's second law. As we noted earlier, diffusion in a solution is an important process in the movement of solutes across plant cells and tissues. How rapid are such processes? For example, if we release a certain amount of material in one location, how long will it take before we can detect that substance at various distances? In order to discuss such phenomena adequately, we must determine the dependence of the concentration on both time and distance. We can readily derive such a time-distance relationship if we first consider the concept of continuity, it being necessary if we are to transform Equation 1.1 into an expression convenient for describing the actual solute distributions caused by diffusion. In particular, we want to eliminate J_j from Equation 1.1 so that we can see how c_j depends on x and t .

The flux J_j of some species j is the amount per unit time crossing a given area, here considered to be a planar area perpendicular to the x -axis (Fig. 1.5). In general, J_j can change with position along the x -axis. Let us imagine

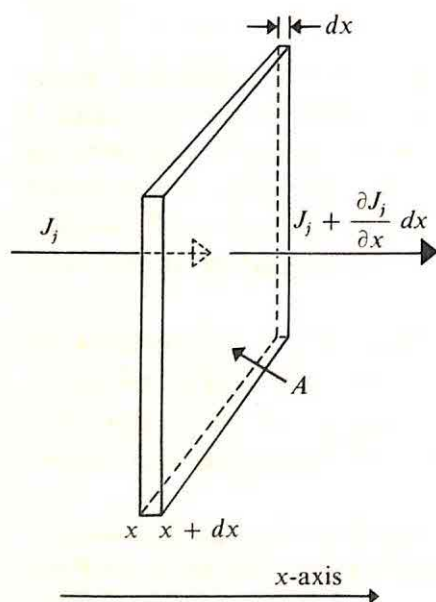


Figure 1.5

Diagram showing the dimensions and fluxes which form the geometrical basis for the continuity equation.

a volume element of thickness dx in the direction of flow and of cross-sectional area A (Fig. 1.5). At x , we will let the flux across the surface of area A be J_j . At $x + dx$, the flux has changed to $J_j + (\partial J_j / \partial x) dx$, where $\partial J_j / \partial x$ is the gradient of the flux of j in the x -direction, i.e., the rate of change of J_j with position, $\partial J_j / \partial x$, times the distance, dx , gives the overall change in the flux, $(\partial J_j / \partial x) dx$. The change in the amount of species j in the volume element $A dx$ in unit time for this one-dimensional problem is simply the amount flowing in per unit time, $J_j A$, minus that flowing out, $[J_j + (\partial J_j / \partial x) dx] A$. The change in the amount of species j in the volume element in unit time can also be expressed in another way: the product of the rate of change in the concentration of species j with time, $\partial c_j / \partial t$, times the volume element in which the change in concentration occurs, $A dx$. Equating these two different expressions that describe the change in the amount of species j in the volume element $A dx$, we obtain the following relation:

$$J_j A - (J_j + \frac{\partial J_j}{\partial x} dx) A = \frac{\partial c_j}{\partial t} A dx \quad (1.2)$$

The two $J_j A$ terms appearing on the left side of Equation 1.2 cancel each other. After dividing through by $A dx$, Equation 1.2 leads to the very useful expression known as the *continuity equation*:

$$-\frac{\partial J_j}{\partial x} = \frac{\partial c_j}{\partial t} \quad (1.3)$$

The continuity equation is a mathematical way of stating that matter cannot be created or destroyed under ordinary conditions. Thus, if the flux of some species decreases as we move in the x -direction ($\partial J_j / \partial x < 0$), Equation 1.3 indicates that its concentration must consequently be increasing with time, as the material is then accumulating locally. When we substitute Fick's first law (Eq. 1.1) into the continuity equation (Eq. 1.3), we obtain *Fick's second law*. For the important special case of constant D_j , this general equation for diffusion becomes

$$\frac{\partial c_j}{\partial t} = -\frac{\partial}{\partial x} \left(-D_j \frac{\partial c_j}{\partial x} \right) = D_j \frac{\partial^2 c_j}{\partial x^2} \quad (1.4)$$

The solution of Equation 1.4 describes how the concentration of some solute changes with position and time as a result of diffusion. In order to determine the particular function which satisfies this important partial differential equation, we need to know the special conditions for the specific problem under consideration. Nevertheless, a representative solution useful

for the consideration of diffusion under simple initial and boundary conditions will be sufficient for the present purpose of describing the characteristics of solute diffusion in general terms. For example, we will assume that there are no obstructions in the x -direction and that species j is initially placed in a plane at the origin ($x = 0$). In this case, the following expression for the concentration of species j satisfies the differential form of Fick's second law when D_j is constant, and also satisfies our rather simple conditions: *

$$c_j = \frac{M_j}{2(\pi D_j t)^{1/2}} e^{-x^2/4D_j t} \quad (1.5)$$

In Equation 1.5 M_j is the total amount of solute j per unit area initially ($t = 0$) placed in a plane located at the origin of the x -direction (i.e., at $x = 0$, while y and z can have any value, which defines the plane considered here). The quantity c_j is its concentration at position x at any later time t . In order for M_j to have this useful meaning, the factor $1/[2(\pi D_j)^{1/2}]$ is necessary in Equation 1.5.† Moreover, the solute in this case is allowed to diffuse for an unlimited distance in either the plus or minus x -direction and no additional solute is added at times $t > 0$. Often this idealized situation can be achieved by inserting a radioactive tracer in a plane at the origin of the x -direction. Equation 1.5 is only one of the possible solutions to the second order partial differential equation representing Fick's second law. The form is relatively simple, however, compared with other solutions, and the initial condition of having a finite amount of material released at a particular location is actually realistic for certain applications to biological problems.

Time-distance relation for diffusion. Although the functional form of c_j given by Equation 1.5 is only one particular solution to Fick's second law (Eq. 1.4) and is restricted to the case of constant D_j , nevertheless, it proves to be an extremely useful expression for understanding diffusion. It relates

* To show that Equation 1.5 is indeed a possible solution of Fick's second law, it can be substituted into Equation 1.4 and the differentiations performed (M_j and D_j are constant; $\partial ax^n/\partial x = anx^{n-1}$, $\partial e^{ax^n}/\partial x = anx^{n-1} e^{ax^n}$, and $\partial uv/\partial x = u\partial v/\partial x + v\partial u/\partial x$, as indicated in App. VI). The solution of Equation 1.4 becomes progressively more difficult when more complex initial and boundary conditions or molecular interactions (which cause variations in D_j) are considered. (See Crank and Jacobs for analytical solutions in such cases.)

† Note that $\int_{-\infty}^{\infty} c_j(x,t) dx = M_j$, where $c_j(x,t)$ is the concentration function which depends on position and time as given by Equation 1.5 and the probability integral, $\int_{-\infty}^{\infty} e^{-a^2 u^2} du$, equals $\sqrt{\pi}/a$.

the distance a substance diffuses to the time necessary to reach that distance. The expression involves the diffusion coefficient of species j , D_j , which can be determined experimentally. In the plane at the origin of the x -direction ($x = 0$), Equation 1.5 indicates that the concentration is $M_j/[2(\pi D_j t)^{1/2}]$, which becomes infinitely large as t is turned back to 0, the starting time. This infinite value for c_j at $x = 0$ corresponds to having all the solute initially placed in a plane at the origin. For t greater than 0, the material begins to diffuse away from the origin. The distribution of molecules along the x -axis at two successive times is indicated in Figure 1.6a and 1.6b, while Figure 1.6c explicitly shows the movement of the concentration profiles along the time axis. Since the total amount of species j does not change (it remains at M_j per unit area of the y - z plane, i.e., in a volume element parallel to the x -axis and extending from x values of $-\infty$ to $+\infty$), the area under each of the concentration profiles is the same.

Comparing Figure 1.6a with 1.6b, we see that the average distance of the diffusing molecules from the origin increases with time. Also, Figure 1.6c shows how the concentration profiles flatten out as time increases, since the diffusing species is then distributed over a greater region of space. In estimating how far molecules diffuse in time t , a useful parameter is the distance at which the concentration has dropped to $1/e$ or 37% of its value in the plane at the origin. Although somewhat arbitrary, this parameter describes the shift of the statistical distribution of the molecules with time. From Equation 1.5, the concentration at the origin is $M_j/[2(\pi D_j t)^{1/2}]$. (See also Fig. 1.6.) The concentration drops to 37% of the value at the origin when the exponent of e in Equation 1.5 is -1 . From Equation 1.5, this distance, x_e , is therefore given by

$$x_e^2 = 4D_j t \quad (1.6)$$

The distance x_e along the x -axis is also indicated in Figures 1.6a and 1.6b.

Equation 1.6 is an extremely important relationship that indicates a fundamental characteristic of diffusion processes: the distance a population of molecules of a particular species diffuses—for the one-dimensional case where the molecules are released in a plane at the origin—is proportional to the square root of both the diffusion coefficient of the species and the time for diffusion. In other words, the time for diffusion increases with the square of the distance to be traversed. It should be stressed that an individual molecule may diffuse a greater or lesser distance in time t than is indicated by Equation 1.6 (cf. Fig. 1.6), since the latter refers to the time required for the concentration of species j at position x_e to become $1/e$ of the value at the origin. Furthermore, the factor 4 is rather arbitrary, since

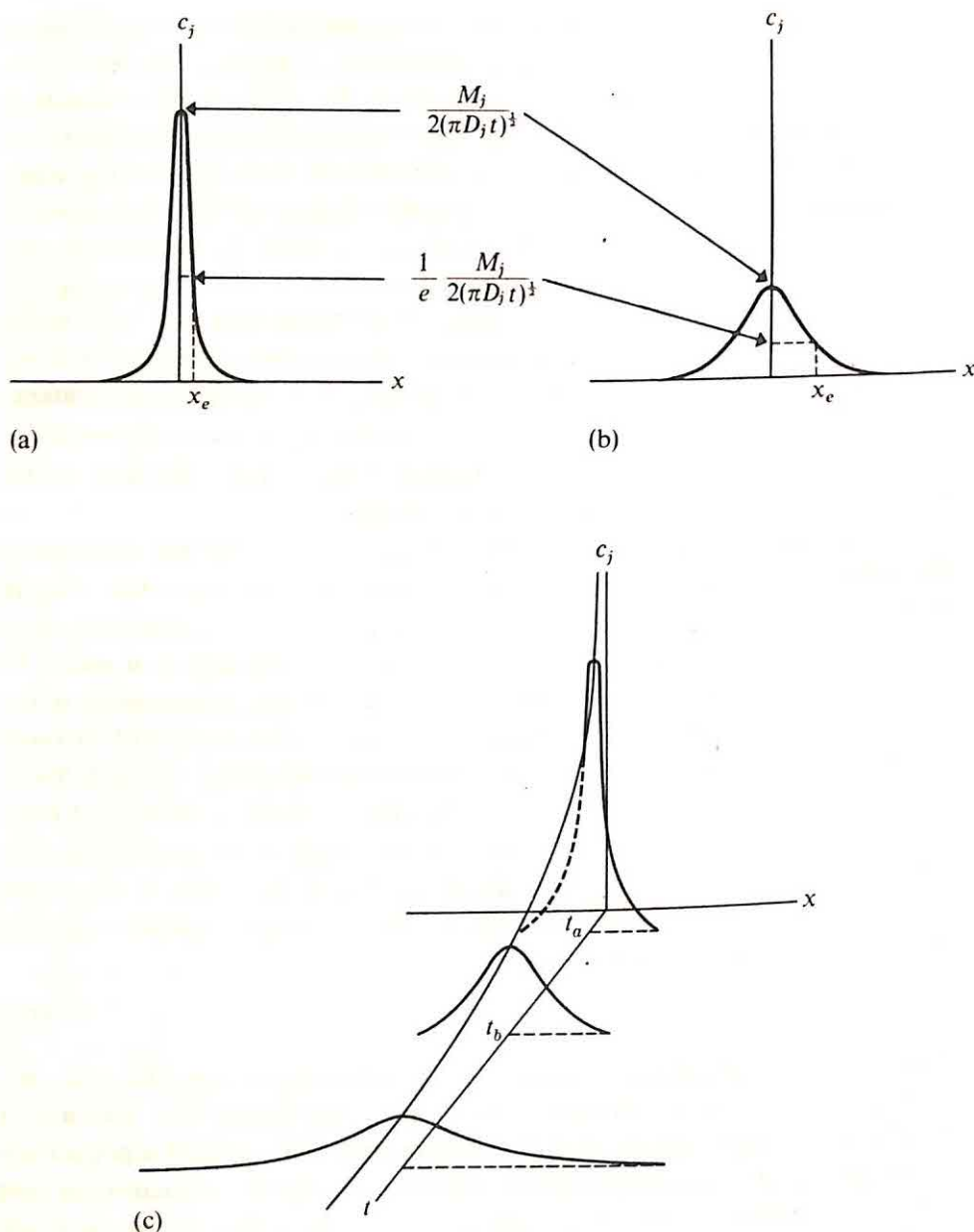


Figure 1.6

Concentration of species j , c_j , as a function of position x for molecules diffusing according to Fick's second law. The molecules were initially placed in a plane at the origin of the x -direction, i.e., at $x = 0$. For a given value of x , c_j is the same throughout a plane in the y - and z -directions. (a) Distribution of concentrations along the x -axis occurring at a time t_a near $t = 0$ (b) distribution occurring at a subsequent time t_b (c) portrayal of the concentration profiles as a function of time. Note that x_e is the location where the concentration of species j has dropped to $1/e$ of its value at the origin.

some criterion other than $1/e$ would cause this numerical factor to be somewhat different, although the basic form of Equation 1.6 would be preserved.

We will now consider the magnitude of D_j for solutes in water. Some diffusion coefficients determined for 10 mM solutions at 25° are as follows:* sucrose, 0.52×10^{-5} cm²/sec; glucose, 0.67×10^{-5} cm²/sec; glycine, 1.1×10^{-5} cm²/sec; calcium (as the chloride), 1.2×10^{-5} cm²/sec; and potassium (as the chloride), 1.9×10^{-5} cm²/sec (see Weast). Hence, the diffusion coefficients of small ions and other molecules in aqueous solutions are approximately 10^{-5} cm²/sec. A globular protein with a molecular weight of 15,000 might have a diffusion coefficient near 10^{-6} cm²/sec at 20°, while D_j for a 10^6 molecular weight globular protein is usually close to 2×10^{-7} cm²/sec. Fibrous proteins often have approximately twofold lower diffusion coefficients than do globular proteins of the same molecular weight. The lower value of D_j for fibrous proteins is due to the greater frictional interaction between water molecules and fibrous proteins compared with the more compact globular ones. (See Sober and Tanford for data on various D_j s of proteins.)

To illustrate the time-distance consequences of Equation 1.6, we will quantitatively consider the diffusion of small molecules in an aqueous solution. How long, on the average, would it take a small solute with a D_j of 10^{-5} cm²/sec to diffuse 50 μ m, the distance across a typical leaf cell? From Equation 1.6, the time required for the population of molecules to shift this distance is $(50 \times 10^{-4} \text{ cm})^2 / [(4)(10^{-5} \text{ cm}^2/\text{sec})]$ or 0.6 sec. Thus, diffusion is a fairly rapid process over subcellular distances. (In the cytoplasm of living cells there is a mechanical mixing due to *cytoplasmic streaming*, which can lead to even more rapid movement than by diffusion.) Next, let us consider the diffusion of the same substances over a distance of 1 meter. The time needed is $(100)^2 / [(4)(10^{-5})]$ or 2.5×10^8 sec, which is eight years! Diffusion is indeed not a rapid process over long distances. Thus, inorganic nutrients in xylary sap would not ascend a tree by diffusion at a rate necessary to sustain life. On the other hand, diffusion is often sufficient for the movement of solutes within leaf cells and especially inside organelles such as chloroplasts and mitochondria. In summary, diffusion in a solution is fairly fast over short distances (less than about 100 μ m) but extremely slow for very long distances.

*The symbol ° in this text represents degrees on the Celsius temperature scale. By recent international agreement, the Celsius degree is $1/273.16$ of the thermodynamic temperature of the triple point of water (0.01000°) and absolute zero is at -273.15° . Although it is obviously still in use, the term "centigrade" is no longer recommended by the international commission on nomenclature.

Diffusion of gases in the air surrounding and within leaves is necessary for both photosynthesis and transpiration. For instance, water vapor evaporating from the cell walls of leaf cells diffuses across the intercellular air spaces (Fig. 1.2) to reach the stomata and from there diffuses across the unstirred air layer into the atmosphere (considered in detail in Ch. 7). CO_2 diffuses from the atmosphere through the open stomata to the surfaces of leaf cells while the photosynthetically evolved O_2 traverses the same pathway in the reverse direction, also primarily by diffusion. The experimentally determined diffusion coefficients of these three gases in air at 20° are approximately as follows: for water vapor, $0.25 \text{ cm}^2/\text{sec}$; for carbon dioxide, $0.16 \text{ cm}^2/\text{sec}$; and for oxygen, $0.20 \text{ cm}^2/\text{sec}$. The diffusion coefficients in air are approximately 10^4 times greater than the D_j describing the diffusion of a solute in a liquid, indicating that diffusion coefficients depend markedly on the medium. In particular, many more intermolecular collisions occur per unit time in a liquid phase than in a less dense gas phase. Thus, a molecule can diffuse further in air than in an aqueous solution before being influenced by other molecules. Most cells in animals are bathed by fluids, so that both a hemoglobin-type molecule and a circulatory system are necessary in order to transport O_2 to their cells and CO_2 away. Plants, on the other hand, often have conspicuous intercellular air spaces by which they can take advantage of the large values of D_{O_2} and D_{CO_2} in a gas phase.

As indicated by Graham's law, the diffusion coefficients of gases in air are generally inversely proportional to the square root of the molecular weights. This proportionality also often holds for chemically related small substances in solution. (For large substances, the proportionality is often with the cube root of molecular weight—see Bull and Stein.) The molecular weight of H_2O is 18, and for CO_2 it is 44. Water vapor therefore should have a diffusion coefficient $\sqrt{(44)/(18)}$, or 1.56 times higher than that of CO_2 , as the above values for D_j in air indicate. In addition to this dependence of D_j on molecular weight and, by implication, on molecular size, the diffusion coefficient depends inversely on the viscosity of the medium (discussed on p. 105, where the temperature dependence of D_j is also considered).

Because of the complicated anatomy of a leaf, the actual pathway for the movement of gas molecules in the intercellular air spaces can be rather tortuous. Nevertheless, calculations using Equation 1.6 can give useful estimates of the diffusion times for the many processes involving gaseous movements in a leaf. An upper limit for the diffusion pathway in such intercellular air spaces of a leaf might be $1000 \mu\text{m}$. Using Equation 1.6 and the diffusion coefficients given in the previous paragraph, we can calculate that the times needed to diffuse $1000 \mu\text{m}$ for water vapor, carbon dioxide, and oxygen in air are from 10 to 16 milliseconds. This illustrates that

diffusion of molecules in a gas is a fairly rapid process. However, when illumination and temperature are not limiting, the rate of photosynthesis in plants is generally limited by the amount of CO_2 diffusing up to the chloroplasts, while the rate of transpiration is controlled by diffusion of water vapor from the cell walls within the leaf to the outside air. In the latter case, the diffusion limitation helps prevent excessive water loss from the plant and therefore is quite useful, physiologically speaking.

Membrane structure

The plasmalemma presents a major barrier to the diffusion of solutes into and out of plant cells, while the organelle membranes play an analogous role for the various subcellular compartments and the tonoplast for the vacuole. For instance, although H_2O and CO_2 readily penetrate the plasmalemma, ATP and metabolic intermediates usually do not diffuse across it at an appreciable rate. Before a mathematical description of the penetration of membranes by solutes, we need to briefly review certain features of the structure of membranes.

Membrane models. A general model for membrane structure is the bimolecular lipid leaflet flanked on either side by protein layers (Fig. 1.7a). Gorter and Grendel in 1925 estimated that the area covered by the lipids extracted from erythrocytes (red blood cells), when spread as a monomolecular layer on water, was about twice the surface area of the cells (the relative area of the lipids is actually somewhat less than they deduced). The amount of lipid present was apparently sufficient to form a double layer in the membrane. Moreover, the penetration of a series of species across membranes often appeared to depend primarily on the relative lipid solubility of the molecules (see Davson and Danielli; Diamond and Wright; and Stein). This circumstantial evidence eventually led to the concept of a lipid bilayer presumed to be located in the middle of biological membranes.

To help understand the resulting membrane models, we must consider the charge distribution within lipid molecules. The arrangement of atoms in the hydrocarbon (containing only C and H) region of lipid molecules leads to bonding in which no local charge imbalance develops. This hydrocarbon part of the molecule is thus nonpolar and tends to avoid water, and is therefore called *hydrophobic*. Most lipid molecules in membranes also have a phosphate and/or an amine group. This region of the molecule becomes charged in aqueous solutions. Such charged regions interact electrostatically with the polar parts of other molecules. Since they interact attractively with water, the polar regions are termed *hydrophilic*.

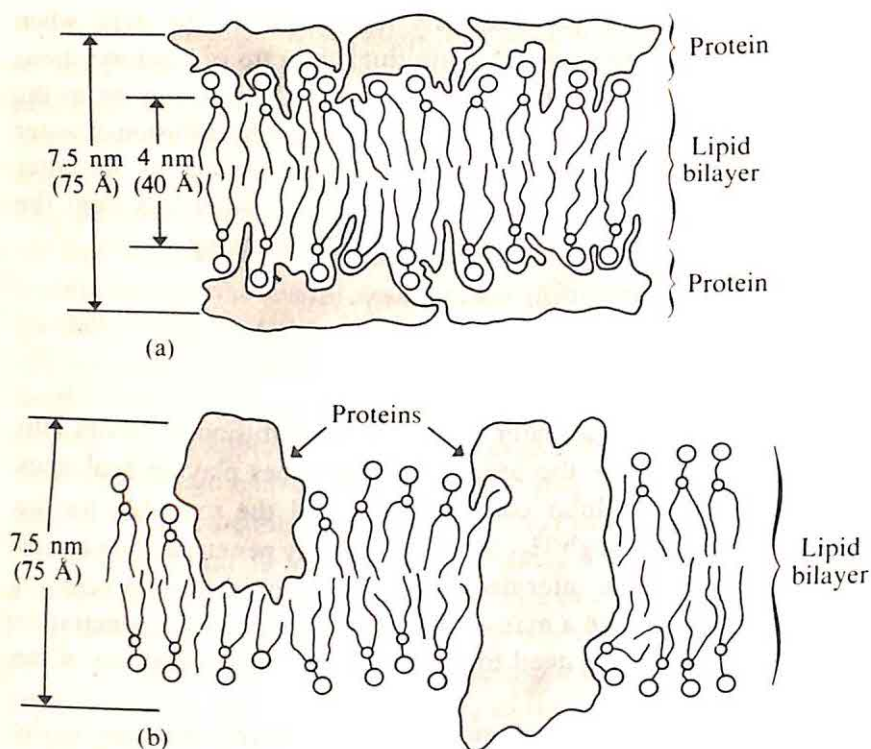
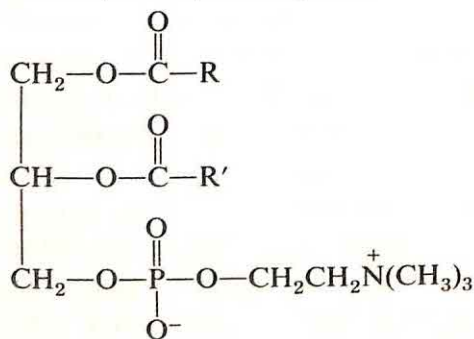


Figure 1.7

Membrane models: (a) bimolecular lipid leaflet with protein layers adsorbed on both sides (b) globular proteins interspersed within lipid bilayer. The ionic “head” of phospholipids is represented by $\bigcirc-\bigcirc$, while the fatty acid side chains leading to the nonpolar “tail” are indicated by the two wavy lines emanating from the head.

Many of the phospholipids in membranes are glycerol derivatives that have two esterified fatty acids plus a charged side chain joined by a phosphate ester linkage. A typical example is phosphatidyl choline (also known by the older name *lecithin*), a major component of most membranes:



where R and R' are the hydrocarbon parts of the fatty acids. Various fatty acids which are commonly esterified to this phospholipid include palmitic (16C and no double bonds, represented as 16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3). (The major fatty acid combined in various forms in the membranes of higher plant chloroplasts is linolenic.) The hydrocarbon side chains from the esterified fatty acids affect the packing of the lipid molecules in a membrane. For instance, as the number of double bonds in the fatty acid side chain increases, the side chain tends to be less straight, so that the area per lipid molecule in a monolayer becomes greater. This change in intermolecular distances affects the permeability of such lipid layers as well as, presumably, that of biological membranes.

To form a bilayer, the lipid molecules could have their nonpolar portions adjacent to each other (Fig. 1.7), facilitating hydrophobic interactions. The polar or hydrophilic regions are then on the outside. These outer charged regions of the lipids can attract water molecules and/or the charged parts of proteins, both of which are present in membranes. For instance, up to 70% of the dry weight of mitochondrial membranes may be protein. The ratio of protein to lipid by weight is generally 2 to 3 for most membranes. Membranes also contain a small amount of oligosaccharides, often bound to proteins.

Davson and Danielli proposed that the membrane proteins were adsorbed as monolayers on either side of the central lipid bilayer. Most proteins are fairly heavily charged in aqueous solutions and could interact with both the polar end of the lipids and the surrounding water molecules in an aqueous solution. The model (Fig. 1.7a) is consistent with the apparent presence of a lipid phase and the substantial protein content of membranes. A lipid bilayer with protein monolayers adsorbed on both sides would be approximately 7 to 8 nm or 70 to 80 Ångstroms thick. (An Ångstrom, abbreviated Å, is 10^{-8} cm. Thus, 1 nm is 10 Å.) Lipid bilayers by themselves are 4 to 5 nm thick, while each protein monolayer would be 1 to 1.5 nm in thickness. In electron micrographs of thin sections through cells, many membranes have two regions of heavy metal deposition about 2 nm wide on either side of a relatively clear space about 3.5 nm wide. These electron-dense bands were proposed to be protein layers, the unstained central region of the membrane to be a lipid region. However, the Davson-Danielli model leads to certain discrepancies which we must now consider.

Both thermodynamic predictions and many experimental results suggest that membrane proteins do not occur as monolayers, but rather in a globular form imbedded within the membranes (see Singer and Nicolson). For



instance, the α -helical content of proteins in membranes is consistent with that of globular proteins, but far greater than that expected for monolayers. If globular proteins were attached to the outer surfaces of a lipid bilayer, the thickness would be greater than that observed for membranes. Proteins may thus occur as globular subunits embedded within a lipid matrix, as in Figure 1.7b, and indeed some proteins may extend all the way across the membrane. This leads us to a reconsideration of the hydrophobic and hydrophilic forces within a membrane. In particular, certain globular proteins apparently have their hydrophobic portions buried within the membrane, while a hydrophilic (polar or charged) part sticks out into the aqueous solution next to the membrane. Amino acids whose side chains dissociate—*aspartate*, *glutamate*, *arginine*, *lysine*—tend to be exposed to water, while amino acids with hydrophobic side chains—*leucine*, *isoleucine*, *valine*—tend to the interior of the membrane where they interact with the fatty acid side chains of the phospholipids, which are also hydrophobic. In this model the lipid still occurs as a bilayer, but the hydrophilic portions of the lipids interact directly with water on either side of the membrane (Fig. 1.7b). In the Davson-Danielli model the nonpolar side chains of the amino acids in the protein monolayers tend to be exposed to water, a situation which is thermodynamically less stable than in the globular model, while at the same time the hydrophilic interaction between phospholipids and water is partially screened by the protein layer. Both models (Fig. 1.7) indicate that the main barrier to permeability is the lipid bilayer, a fact consistent with experimental observations.

Organelle membranes. Both mitochondria and chloroplasts have extensive internal membrane systems, and both are highly involved with cellular metabolism. When membranes of such organelles are carefully investigated with electron microscopy, a globular substructure is indeed found (see Park and Sane). For example, particles with major axes near 10 nm and 17 nm have been observed within the lamellar membranes of chloroplasts using the freeze-etch technique—the frozen specimens are fractured, water is removed by sublimation, and electron microscopy is then performed using suitable replicas. These subunits appear to be embedded in the membrane, not adsorbed as monolayers on the surface.

Proteins implementing electron transfer in respiration and in photosynthesis are found in the interior membranes of mitochondria and chloroplasts, respectively. Such membrane subunits are able to thermally move or vibrate due to their own kinetic energy while remaining in the membrane. In fact, diffusion of globular proteins *within* the plane of the membrane has been observed. Diffusion coefficients are somewhat less than 10^{-10} cm²/sec

compared with nearly 10^{-6} cm²/sec for the same proteins in solution, suggesting that the membrane has a rather high viscosity (see Singer and Nicolson). Such diffusion of membrane proteins could allow successive interactions of some bound substrate with various enzymes located in the membrane. Furthermore, the side-by-side location of various components involved with electron transfer in a semi-solid part of the membrane system could ensure an orderly, rapid, directed passage of electrons from enzyme to enzyme.

The proteins involved with electron transfer vary in size and shape, and thus the internal membranes of chloroplasts and mitochondria would not be expected to be uniform and regular. Proteins taking part in ion transport and possibly cell wall synthesis may be embedded in the plasmalemma, while other proteins involved with transport also presumably occur in the tonoplast. On the other hand, many globular proteins in membranes could serve a simply structural role. Between the globular proteins there may be a lipid bilayer arrangement (as illustrated in Fig. 1.7b), which could account for the correlation between lipid solubility and membrane permeation noted above.

Of the two mitochondrial membranes (Fig. 1.8), the outer one appears to be rather permeable to sucrose, various small anions and cations, adenine nucleotides, coenzyme A, and many other compounds, in contrast with the much less permeable inner mitochondrial membrane. The inner membrane apparently invaginates to form the mitochondrial *cristae*, in which are embedded the enzymes responsible for electron transfer and the accom-

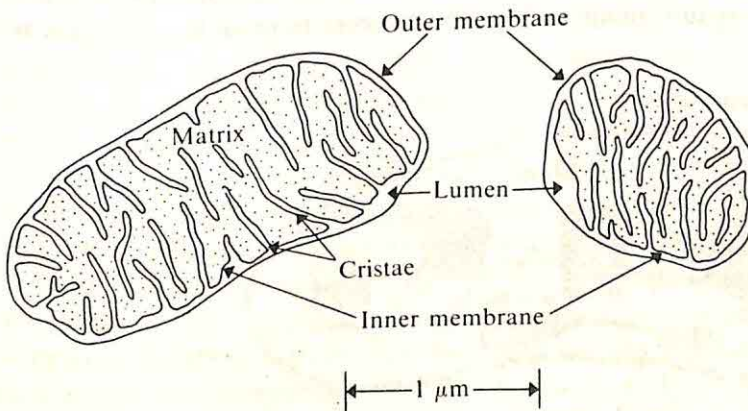


Figure 1.8

Representative mitochondria as seen in an electron micrograph of a 50 nm (500 Å) thick section of plant tissue.

panying ATP formation. For instance, the inner membrane system contains various dehydrogenases, an ATPase, and cytochromes *a*, *a*₃, *b*, and *c*. (Cytochromes are discussed in Chs. 5 and 6.) These proteins with enzymatic activity are not in a simple flat monolayer adsorbed onto a lipid bimolecular leaflet, rather they occur in a globular form. Electron microscopy has revealed small particles attached by stalks to the cristae, indicating another divergence from the simple Davson-Danielli model of a membrane (see Green and Baum as well as Rothfield). These structures embedded in, or released from the mitochondrial membranes are believed to be involved in the phosphorylation accompanying respiration.

Inside the inner membrane of a mitochondrion is a viscous region known as the *matrix* (Fig. 1.8). The citric acid cycle (Krebs cycle) enzymes as well as others are located there. For substrates to be catabolized via the citric acid cycle, they must cross two membranes to pass from the cytoplasm to the inside of the mitochondrion. Often the slowest or rate-limiting step in the oxidation of such substrates is their entry into the mitochondrial matrix. Since the inner mitochondrial membrane is highly impermeable to most molecules, transport across the membrane using a "carrier" (see p. 133) is generally invoked to explain how various substances get into the matrix. These carriers, situated in the inner membrane, might shuttle important substrates from the lumen between the outer and inner mitochondrial membranes to the matrix. Because of the inner membrane, important ions and substrates in the mitochondrial matrix do not leak out. Consequently, such permeability barriers between various subcellular compartments can improve the overall efficiency of the cell.

Chloroplasts (Fig. 1.9) are also surrounded by two membranes, the inner one of which may invaginate to form the complex internal lamellar system.

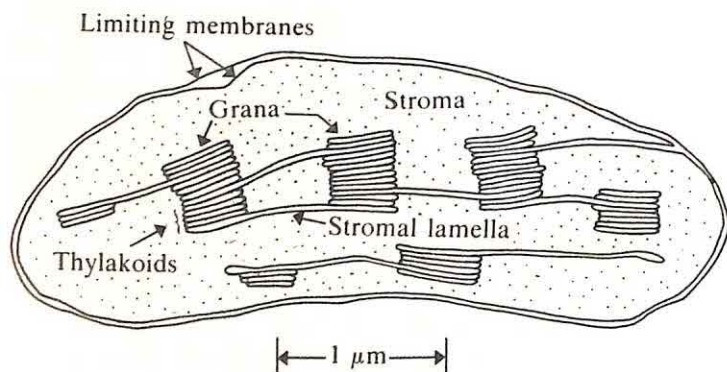


Figure 1.9

Generalized chloroplast from a leaf mesophyll cell.

Although it is obviously important to a full understanding of photosynthesis *in vivo*, only recently has progress been made in understanding the permeability properties and the composition of the two *limiting* membranes (see Douce *et al.* and pp. 154–156). Chloroplast *lamellar* membranes are about half lipid and half protein by dry weight. The chlorophyll and most other photosynthetic pigments are bound to the proteins. These proteins, as well as other components involved with photosynthetic electron transfer (see Chs. 5 and 6), are anchored in the lamellar membranes, apparently by hydrophobic forces (cf. Fig. 1.7b). Each of the lamellae consists of a pair of closely apposed membranes 6 to 8 nm thick. In many regions of the chloroplast the lamellae form flattened sacs, which are referred to as *thylakoids*, or discs (Fig. 1.9). When seen in an electron micrograph, a transverse section of a thylakoid shows a pair of apposed membranes joined at the ends.

The actual organization of lamellar membranes within the chloroplast varies greatly with the species. The chloroplasts of red algae appear to have the simplest internal structure, since the lamellae are in the form of single large thylakoids separated by appreciable distances from each other. For most higher plant chloroplasts, the characteristic feature is stacks of about 10 or more thylakoids known as *grana* (Fig. 1.9), which are typically 0.4 to 0.5 μm in diameter, 10 to 50 of these grana occurring in a single chloroplast. The lamellar extensions between grana are called intergranal or stromal lamellae. The remainder of the chloroplast volume is known as the *stroma*, containing the enzymes involved with the fixation of CO_2 into the various products of photosynthesis. The blue-green algae and the photosynthetic bacteria do not contain chloroplasts, but their photosynthetic pigments are also generally located in membranes, often in lamellae immediately underlying the cell membrane. In some photosynthetic bacteria the lamellae appear to have pinched off and formed discrete subcellular bodies sometimes referred to as *chromatophores*.

“Microbodies” are also quite numerous in plant cells. They are generally divided into two classes, glyoxysomes and peroxisomes, the latter being about three times as prevalent as mitochondria in many leaf cells. Microbodies are usually spherical and from 0.3 to 1.2 μm in diameter. They differ from mitochondria and chloroplasts in that they are surrounded by a single membrane. Both glyoxysomes and peroxisomes carry out only a portion of an overall metabolic pathway, therefore these subcellular compartments are interdependent on reactions in the cytoplasm and in other organelles. In Chapter 7 we will briefly consider the role of peroxisomes in photorespiration.

Membrane permeability

With this knowledge of the general structure of membranes, let us now turn to a quantitative analysis of the interactions between membranes and diffusing solutes. The rate-limiting step for movement of many molecules into and out of plant cells is diffusion through the plasmalemma. Because of the close packing and the interactions between the component molecules of a membrane, such diffusion is greatly restricted compared with the relative ease of movement in an aqueous phase like the cytoplasm, i.e., the solute has great difficulty in threading its way between the molecules composing the membrane. The average diffusion coefficient of a small solute in a membrane is often about 10^6 times lower than in the adjacent aqueous solutions. Also, the membrane represents a different type of molecular environment than does an aqueous solution, so that the solubility of a species in the two phases must be taken into account in order to describe membrane permeability. As will be shown, Fick's first law can be modified to describe the diffusion of molecules across a membrane. Once past the barrier presented by the membrane, the molecules may be distributed throughout the cell relatively rapidly by diffusion, and over greater distances by cytoplasmic streaming.

Concentration difference across a membrane. The driving force for diffusion of uncharged molecules into or out of plant cells can be regarded as the negative of the concentration gradient of that species across the plasmalemma. Since the actual concentration gradient of some species j is not known in the plasmalemma (or in any other membrane for that matter), the driving force is generally approximated by the negative of the average gradient of that species across the membrane:

$$-\frac{\partial c_j}{\partial x} \cong -\frac{\Delta c_j}{\Delta x} = -\frac{c_j^i - c_j^o}{\Delta x} = \frac{c_j^o - c_j^i}{\Delta x} \quad (1.7)$$

where c_j^o is the concentration of species j outside the cell, c_j^i is its concentration in the cytoplasm, and Δx is the thickness of the plasmalemma, which acts as the barrier restricting the penetration of the molecules into the cell (see Fig. 1.10). The concentrations c_j^o and c_j^i appearing in Equation 1.7 can also represent values in the phases separated by any other membrane, such as the tonoplast. If c_j^i is less than c_j^o as we move in the positive x -direction (cf. Fig. 1.10), $\partial c_j / \partial x$ is then negative and so $-\partial c_j / \partial x$ is positive; the flux J_j would then occur in the positive x -direction (cf. Eq. 1.1, $J_j = -D_j \partial c_j / \partial x$). Also, if we designate a net flux into a cell as a positive flux—which is the commonly used convention—then the minus sign in $-\partial c_j / \partial x$ is incorporated

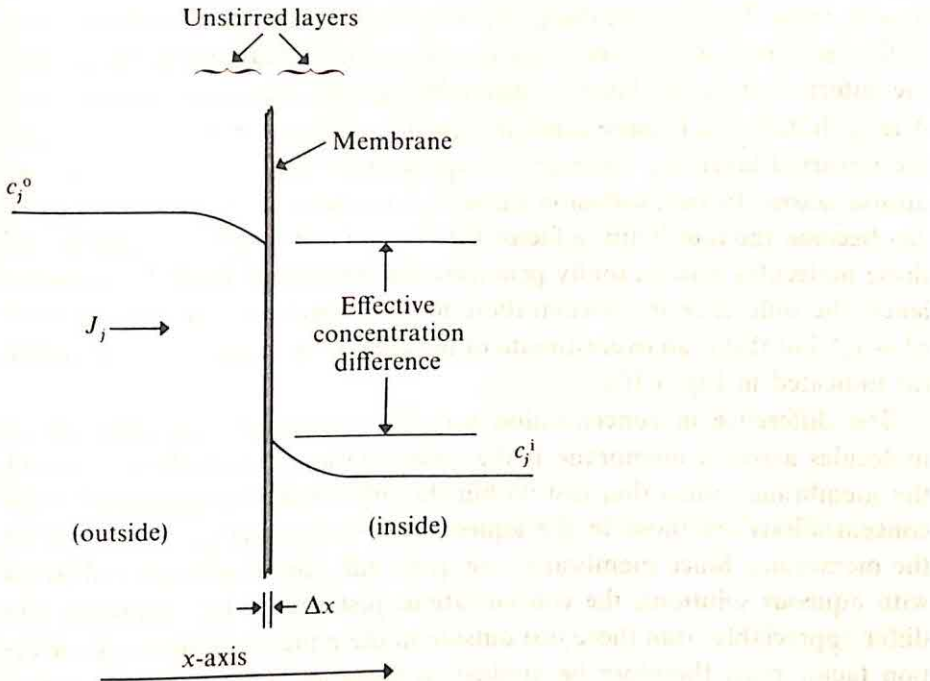


Figure 1.10

The effect of unstirred layers on the concentration of species j near a membrane across which the solute is diffusing.

into the concentration difference ($c_j^o - c_j^i$) that we must use to describe the flux, viz., $J_j = D_j(c_j^o - c_j^i)/\Delta x$.

The magnitude of what may be called the “effective” concentration difference across a membrane is made somewhat uncertain by the existence of unstirred boundary layers (Fig. 1.10), as has been pointed out by J. Dainty (see Dainty). Boundary layers in which there is no turbulent mixing occur at the interface between any fluid (liquid or gas) and a solid, such as the unstirred air layers on either side of a leaf (discussed in Ch. 7). A substance moves across these unstirred layers next to the membrane or other solid not by mechanical mixing but by diffusion, indicating that a concentration gradient must also exist in the boundary layers (cf. Fig. 1.10). When the mixing in the solutions adjacent to a membrane is increased—for example, either by the turbulence resulting from cytoplasmic streaming on the inside of the cell or rapid stirring on the outside—the thicknesses of the unstirred layers are reduced. They are not eliminated however. Under actual experimental conditions with vigorous stirring the external unstirred layer

may be from 10 to 100 μm thick, which is much thicker than the membrane itself (see Green and Otori). Due to cytoplasmic streaming in plant cells, the internal unstirred layer is generally thinner than the external one. Although diffusion is more rapid in aqueous solutions than in membranes, the unstirred layers do represent an appreciable distance for molecules to diffuse across. In fact, diffusion through the unstirred layer in some cases can become the rate-limiting factor for the entry into cells or organelles of those molecules which rapidly penetrate the membrane itself. For convenience, the difference in concentration across a membrane will be taken as $c_j^i - c_j^o$, but this is an overestimate of the effective concentration difference (as indicated in Fig. 1.10).

The difference in concentration actually determining the diffusion of molecules across a membrane is the concentration just inside one side of the membrane minus that just within the other side. In Equation 1.7 the concentrations are those in the aqueous phases outside on either side of the membrane. Since membranes are quite different as solvents compared with aqueous solutions, the concentrations just inside the membrane can differ appreciably from those just outside in the aqueous solution. A correction factor must therefore be applied to Equation 1.7 to give the actual concentration gradient existing in the membrane. This factor is known as the *partition coefficient*, K_j . It is defined as the ratio of the concentration of a solute in the material of the membrane to that in equilibrium outside it in the aqueous phase (c_j^o or c_j^i). As a guide to the situation in the membrane, the partition coefficient is generally determined by measuring the ratio of the equilibrium concentration of some solute in a lipid phase—such as olive oil, which mimics the membrane lipids—to the concentration in an adjacent and immiscible aqueous phase (which mimics the solutions on either side of the membrane). This rather simple convention for obtaining partition coefficients is based on the high lipid content of membranes and the numerous experimental results which show that the relative ease of penetration of a membrane depends on the lipid solubility of the molecules (see Davson and Danielli as well as Diamond and Wright). Partition coefficients have a wide range of values, most being between 10^{-6} and 10 (see Wright and Diamond, where the effect on K_j of various lipids is described). For example, an inorganic ion might have a K_j near 10^{-5} for membranes, while a nonpolar hydrophobic substance might have a value near 1. Usually, K_j is assumed to be the same on the two sides of the membrane. These considerations lead us to write $K_j(c_j^o - c_j^i)$ for the concentration difference leading to diffusion across a membrane, where K_j is characteristic of a particular substance.

Permeability coefficient. We will next use Fick's first law ($J_j = -D_j \partial c_j / \partial x$, Eq. 1.1) to obtain an expression that is convenient for describing the movement of a substance across a membrane. The negative concentration gradient will be replaced by $(c_j^o - c_j^i) / \Delta x$ (Eq. 1.7). Moreover, since D_j is the diffusion coefficient of species j within the substance of the membrane, we must use the actual concentration drop within the membrane, $K_j(c_j^o - c_j^i)$. These various considerations lead us to the following expression describing the diffusion of species j :

$$\begin{aligned} J_j &= D_j \frac{K_j(c_j^o - c_j^i)}{\Delta x} = \frac{D_j K_j}{\Delta x} (c_j^o - c_j^i) \\ &= P_j (c_j^o - c_j^i) \end{aligned} \quad (1.8)$$

where P_j is called the *permeability coefficient* of species j .

Since permeability coefficients have so many applications in physiology, we will define P_j explicitly:

$$P_j = \frac{D_j K_j}{\Delta x} \quad (1.9)$$

which follows directly from Equation 1.8. The permeability coefficient conveniently replaces three quantities which can also be used to describe the diffusion of some solute across a membrane or other barrier. In this regard, the partition coefficients of membranes are generally determined by using a lipid phase like olive oil or ether, not the actual lipids occurring in membranes, and thus any such K_j will have some uncertainty. Also, measurements of Δx and D_j for membranes tend to be rather indirect. On the other hand, P_j is a single, readily measured quantity characterizing the diffusion of some solute across a membrane or other barrier. The units of P_j are length per time, e.g., cm/sec.

Instead of calculating the flux of some species into a cell—the amount entering per unit area per unit time—we often focus our attention on the total amount of that species entering within a certain time interval. Let s_j be the amount of species j inside the cell, where s_j can be expressed in moles. If that substance is not involved in any other reactions, ds_j/dt represents the rate of entry of species j into the cell. The flux J_j is the rate of entry of the substance per unit area or $(1/A)(ds_j/dt)$, where A is the area of the cellular membrane across which the substance is diffusing. Using this expression for the flux, we can express Equation 1.8 as follows:

$$ds_j/dt = J_j A = P_j A (c_j^o - c_j^i) \quad (1.10)$$

When the external concentration of species j (c_j^o) is greater than the internal one (c_j^i), species j will enter the cell and ds_j/dt will then be positive, as Equation 1.10 indicates. This equation is actually very useful for considering many experimental investigations of the diffusion of solutes into and out of plant cells.

Diffusion and cellular concentration. A question that often arises in cellular physiology is how the internal concentration of a penetrating solute changes with time. How long, for example, would it take the internal concentration of an initially absent species to build up to 70% of the external concentration? In order to solve the general case, we must first express s_j in Equation 1.10 in a suitable fashion and then integrate the equation. It is useful to introduce the approximation—particularly appropriate to plant cells—that the cell volume does not change during the time interval of interest. In other words, because of the rigid cell wall, we will assume that the plant cell has a nearly constant volume (V) during the entry or exit of the solute being considered.

The average internal concentration of species j , c_j^i , is equal to the amount of that particular solute inside the cell (s_j) divided by the cellular volume (V), or s_j/V . Therefore, s_j can be conveniently replaced by Vc_j^i , and thus ds_j/dt in Equation 1.10 equals Vdc_j^i/dt when we can assume that the cell volume does not change with time. We are also assuming that species j is not produced or consumed by any reaction within the cell, so that the diffusion of species j into or out of the cell is the only process affecting c_j^i . For simplicity, we are treating the concentration of species j as if it were uniform within the cell. In a more complex case we might need a relation of the form of Equation 1.10 for each membrane-surrounded compartment. Moreover, we are presupposing either mechanical mixing or rapid diffusion inside the cell, so that the drop in concentration ($c_j^o - c_j^i$) occurs essentially only across the membrane. Finally, let us assume that P_j is independent of concentration, a condition that is often satisfactorily met over a limited range of concentration. We can now proceed with the integration of Equation 1.10. Upon replacement of ds_j/dt by Vdc_j^i/dt , rearrangement in order to separate variables (see App. VI), and the insertion of integral signs, Equation 1.10 becomes

$$\int_{c_j^i(0)}^{c_j^i(t)} \frac{dc_j^i}{c_j^o - c_j^i} = \frac{P_j A}{V} \int_0^t dt \quad (1.11)$$

where $c_j^i(0)$ is the initial internal concentration of species j , i.e., when $t = 0$, and $c_j^i(t)$ is that at a later time t .

The volume outside certain unicellular algae and other membrane-

surrounded entities can be quite large compared with V . In such cases the external concentration (c_j^o) does not change appreciably. Such an approximation can also be appropriate for experiments of short duration or where special arrangements are made to maintain c_j^o at some fixed value. For those cases where c_j^o is constant, Equation 1.11 can be integrated to give the following expression: *

$$\frac{P_j A t}{V} = \ln \frac{c_j^o - c_j^i(0)}{c_j^o - c_j^i(t)} \quad (1.12)$$

Starting with a known c_j^o outside a cell and determining the internal concentration both initially, i.e., for $t = 0$, and at some subsequent time t , we can calculate P_j from Equation 1.12 if A/V is known. Even when A/V is not known, the relative permeability coefficients for two substances can be determined. The above derivation can easily be extended to the case where c_j^o is zero. In that case, $P_j A t/V$ equals $\ln [c_j^i(0)/c_j^i(t)]$, an expression that can be used to describe the diffusion of a photosynthetic product out of a chloroplast or some substance from a cell into a large external solution initially devoid of that species. Such studies can be greatly simplified by using radioactive tracers.

When diffusion occurs from all directions across a membrane surrounding a cell (or an organelle), the time to reach a given internal concentration can be directly proportional to the dimensions of the cell. For convenience of calculation in illustrating this point, let us consider a spherical cell of radius r . The volume V then equals $(4/3)\pi r^3$ and the surface area A is $4\pi r^2$. Hence, V/A is $r/3$. In the case of two spherical cells having the same permeability coefficient for some species, Equation 1.12 indicates that the time to reach a given concentration inside is *linearly* proportional to the time to reach a given concentration outside, i.e., t equals $(r/3 P_j) \ln [c_j^o - c_j^i(0)]/[c_j^o - c_j^i(t)]$. For the same P_j , a given level inside a small cell is reached sooner than it would be reached inside a large cell. However, when diffusion across the membrane into the cell occurs from all directions, the ratio of the times to reach a given internal concentration for these two cells equals the ratio of the radii (assuming a uniform c_j^i). On the other hand, the time that it takes for a planar front to diffuse in one direction is proportional to the square of the distance traveled ($x_e^2 = 4D_j t$, Eq. 1.6).

* Recall that $\int dx/(a - x) = -\int d(a - x)/(a - x) = -\ln(a - x) + b$. Also, see Appendix V for information on logarithms and Appendix VI for a detailed consideration of the integration of Equation 1.11.

A typical permeability coefficient for a small nonelectrolyte or uncharged molecule, e.g., isopropanol, phenol, is 10^{-4} cm/sec for the plasmalemma (see Stein) while P_j for a small ion, e.g., K^+ , Na^+ , might be about 10^{-7} cm/sec. The lower permeability coefficients for charged particles are mainly because of the much lower partition coefficients of these solutes compared with nonelectrolytes, which tend to be hydrophobic (recall that $P_j = D_j K_j / \Delta x$, Eq. 1.9). In other words, due to its charge, the electrolyte tends to be much less soluble in a membrane than is a neutral molecule, so that the effective concentration gradient driving the charged species across the membrane is generally smaller for given concentrations in the aqueous phases on either side of the barrier. By comparison, water can rapidly enter or leave cells, and often has a permeability coefficient of about 10^{-2} cm/sec. (Actually, it is hard to measure P_w because of the relative importance of the unstirred layers in this case—see Dainty.)

Using Equation 1.12, we can calculate the time required for a substance initially absent from a cell [$c_j^i(0) = 0$] to achieve an internal concentration equal to half the external concentration [$c_j^i(t) = \frac{1}{2}c_j^o$]. For spherical cells, V/A is $r/3$ as we mentioned above, and the conditions on the concentrations mean that $[c_j^o - c_j^i(0)]/[c_j^o - c_j^i(t)]$ equals $(c_j^o - 0)/(c_j^o - \frac{1}{2}c_j^o)$, which is 2. Substituting these relations into Equation 1.12 indicates that the time needed is $(r/3P_j) \ln 2$, where r is the radius of the spherical cell, P_j is the permeability coefficient of the solute being considered, and $\ln 2$ equals 0.693 (App. III). For a cell 50 μm in diameter—which is a reasonable dimension for spongy mesophyll cells in certain leaves (cf. Fig. 1.2)—the time for an initially absent species to reach half of the external concentration is $\{(25 \times 10^{-4} \text{ cm})/[(3)(10^{-4} \text{ cm/sec})]\} (0.693)$, or 6 sec for a nonelectrolyte with a P_j of 10^{-4} cm/sec, and 1000 times longer (2 hr) for small electrolytes having a P_j near 10^{-7} cm/sec. Hence, the external membrane is an extremely good barrier for electrolytes, markedly hindering their entry into, or egress from the cell. On the other hand, small nonelectrolytes can fairly readily diffuse in and out of plant cells, of course depending to a large extent on their relative lipid solubility.

Cell walls

The rigidity of the cell wall helps determine the size and the shape of the cell and ultimately that of the plant. This supportive role is performed in conjunction with the internal hydrostatic pressure, which causes a distension of the cell walls. The cell wall is also intrinsically involved in many aspects

of the ion and water relations of a plant. Since it surrounds the plasmalemma of each cell, all fluxes of water and solutes into or out of a plant cell protoplast must cross the cell wall, usually by diffusion. Water which evaporates from a plant during transpiration comes directly from cell walls. (Some aspects of this process will be considered quantitatively in Chs. 2 and 7.) The space surrounded by the cell wall in certain specialized cells can act as a channel through which solutions move. In the xylem, for example, the conducting cells have lost their protoplasts with the result that the pathway for the conduction of solutions in the xylem consists essentially of hollow pipes, or conduits, made exclusively of cell walls.

Cell walls vary from tenths to tens of microns in thickness, and generally are divided into three regions. The *primary cell wall* surrounds dividing meristematic cells as well as the elongating cells during the period of cell enlargement. The cell wall often becomes thickened by the elaboration of a *secondary cell wall* inside the primary one (Fig. 1.11), which makes the cell mechanically much less flexible. Hence, cells whose walls have under-

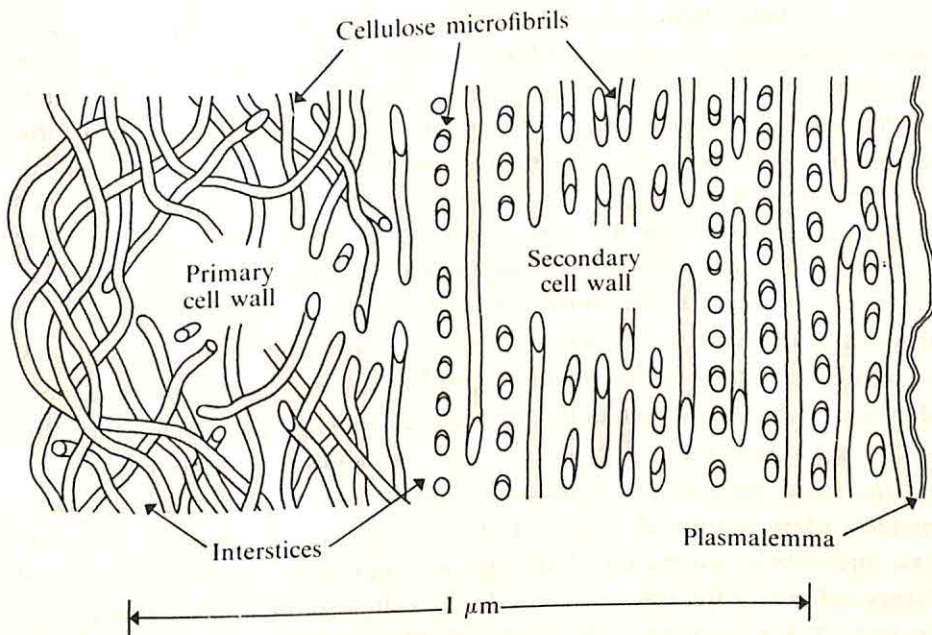
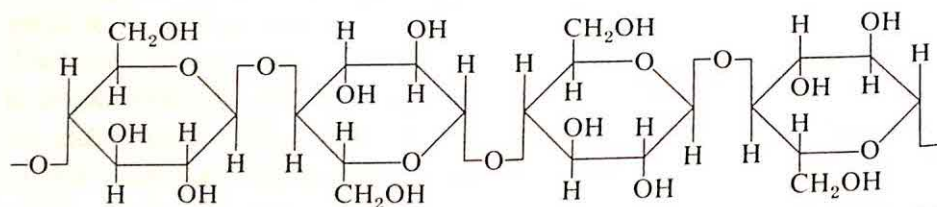


Figure 1.11

Hypothetical thin section through a cell wall, indicating the cellulose microfibrils in the primary and secondary cell walls. The interstices are filled with noncellulosic material including appreciable water, the solvent for solute diffusion across the cell wall.

gone secondary thickening, such as in xylem vessel members, are generally not capable of subsequent elongation. The cell wall also includes an amorphous region between contiguous cells called the *middle lamella*, the extent of which is not universally agreed upon. Although cellulose also occurs there, the middle lamella may be composed mainly of the calcium salts of pectic acids, the presence of which causes adjacent cells to adhere together.

Chemistry and morphology. Cellulose is the most abundant organic component in the entire plant and animal kingdoms. It is the characteristic substance of the plant cell wall, constituting from 25 to 50% of the cell wall organic material. Cellulose is a linear (unbranched) polysaccharide consisting of 1,4-linked β -D-glucopyranose units:



The polymer is about 0.8 nm ($8 \text{ \AA}$) in its maximum width, 0.33 nm^2 ($33 \text{ \AA}^2$) in cross-sectional area, and often contains 8,000 to 12,000 glucose residues. Since the glucose residues have their rings in the same plane, the cellulose polymer is ribbon-like. These polymers are organized into various entities in the cell wall. Approximately 100 parallel chains of cellulose often form a structural unit known as an *elementary microfibril*. Elementary microfibrils can be about 4 nm by 8.5 nm in cross-section. Approximately 20 such elementary microfibrils may be organized in a parallel array into one *microfibril*. The microfibrils are sometimes about 25 nm in diameter and can be $10 \mu\text{m}$ long (see Esau, Northcote, and Siegel for observed variations in the size of microfibrils). Microfibrils are the basic unit of the cell wall and are readily observed in electron micrographs. Although great variation exists, they tend to be interwoven in the primary cell wall and parallel to each other in the secondary wall (cf. Fig. 1.11; also, see Clowes and Juniper).

Interstices between the cellulose microfibrils are usually tens of nanometers (a few hundred $\text{\AA}$) across. In these interstices surrounding the cellulose microfibrils is a matrix of amorphous components which can occupy a larger volume of the cell wall than do the cellulose microfibrils themselves. In fact, on a weight basis, the main constituent of the cell wall is actually water, some of the consequences of which we will consider here and in Chapter 2.

The cell wall matrix contains lignins, noncellulosic polysaccharides such as pectin, a small amount of protein, bound and free water, appreciable

calcium, other cations, and sometimes silicates. Lignins are complex polymers based on phenylpropanoid subunits (a 6-carbon ring, to which is attached a 3-carbon chain as in phenylalanine, a lignin precursor) plus certain other residues. Lignins constitute the second most abundant class of organic molecules in living organisms and are about half as prevalent as cellulose. They are quite resistant to enzymatic degradation and consequently are important in peat and coal formation. Moreover, since the plant itself cannot break down the lignin polymers, cells are unable to expand after extensive lignification of their cell walls, as occurs in the secondary walls of xylem vessel members. (See Sarkanen and Ludwig.) Pectin consists primarily of 1,4-linked α -D-galacturonic acid residues, the carboxyl groups of which are normally dissociated and have a negative charge (galacturonic acid differs from galactose simply by having $-\text{COOH}$ instead of $-\text{CH}_2\text{OH}$ in the 6-carbon position). The negative charge of the dissociated carboxyl groups leads to the tremendous cation-binding capacity of cell walls. In particular, much of the divalent cation calcium (Ca^{++}) is bound, which may help link the various polymers together. Polymers based on 1,4-linked β -D-xylopyranose units (xylans), as well as on many other residues, can also be extracted from cell walls. They are loosely referred to as hemicelluloses, e.g., xylans, mannans, galactans, and glucans. In general, hemicelluloses tend to have a low molecular weight (in the ten thousands) compared with pectin (about five to ten times larger) or cellulose (up to 1000 times larger). (See Bonner and Varner; Clowes and Juniper; Northcote, or Siegel for further details on cell wall chemistry.) The presence of the negatively charged pectins hinders the entry of anions into plant cells. However, as we will consider next, ions and other solutes generally pass through the cell wall much more easily than through the plasmalemma.

Diffusion across cell walls. How would we calculate the ease with which molecules might diffuse across a cell wall? A good place to start is with Equation 1.9, which indicates that the permeability coefficient of species j equals $D_j K_j / \Delta x$. We must first consider what we mean by D_j and K_j in a cell wall. The diffusion of solutes across a cell wall occurs mainly in the water located in the numerous interstices, which are often about 10 nm in diameter. Thus, the movement from the external solution up to the plasmalemma can be in aqueous channels through the cell wall. By the definition of a partition coefficient (solubility in barrier/solubility in water), we recognize that K_j would be unity for the water-filled interstices of the cell wall, it would be very low for the solid phases (cellulose, lignin, and the other polymers present in the wall), and the partition coefficient would have some intermediate value for the cell wall as a whole. Since diffusion takes place

primarily in the cell wall water, we will let K_j be unity. If we use this convenient definition for K_j in cell walls, we must be careful how we define D_j . In particular, diffusion coefficients will have to be expressed on the basis of the whole cell wall, and not on the much smaller area presented by the interstices. The value of D_j in the water of the interstices could be very similar to that in a free solution, while its value averaged over the whole cell wall would be considerably less. Such effective diffusion coefficients of many small solutes are anywhere from ten to one hundred times less when averaged over the cell wall than are the D_j s of the same species in an extended aqueous solution. Thus, the diffusion coefficients we will use here can be as high as about 10^{-6} cm²/sec for small solutes diffusing across cell walls.

Next, let us calculate the permeability coefficient for a solute which has a diffusion coefficient of 10^{-6} cm²/sec for that cell wall. We will assume a representative value of $2\text{ }\mu\text{m}$ for the cell wall thickness. Using Equation 1.9 ($P_j = D_j K_j / \Delta x$), we can thus estimate that P_j for the cell wall might be $[(10^{-6}\text{ cm}^2/\text{sec})(1)]/(2 \times 10^{-4}\text{ cm})$, or 5×10^{-3} cm/sec. Most of the permeability coefficients for small solutes crossing the plasmalemma range from 10^{-8} to 10^{-4} cm/sec. Hence, a cell wall generally has a higher permeability coefficient than does a membrane, which means that the wall is usually more permeable for small solutes than is the plasmalemma. For comparison, let us consider a permeability coefficient that might be appropriate for an unstirred liquid layer adjacent to a cell wall or membrane. Specifically, D_j for a small solute may be 10^{-5} cm²/sec in water, K_j is unity in the aqueous solution and, let us assume, Δx is $30\text{ }\mu\text{m}$ for the unstirred layer. In such a case, P_j would be $(10^{-5})(1)/(30 \times 10^{-4})$ or 3×10^{-3} cm/sec, which is somewhat less than the above value for a cell wall.

Molecules have much greater difficulty diffusing a given distance across the plasmalemma, compared with the same distance in the cell wall or in the adjacent unstirred layer. For the above numerical values, $D_j K_j$ is 10^{-5} cm²/sec in the aqueous solution, 10^{-6} cm²/sec in the cell wall, while for a plasmalemma 10 nm ($100\text{ }\text{\AA}$) thick, $D_j K_j$ is only 10^{-14} to 10^{-10} cm²/sec. Membranes do indeed provide very effective barriers for the diffusion of solutes.

Secondary cell walls are often interrupted by localized pits. In such regions the primary cell walls also have local depressions where they are markedly decreased in thickness. A pit in the wall of a given cell usually occurs opposite a complementary pit in an adjacent cell, the cytoplasm of two adjacent cells being brought into close proximity at such a pit pair. The local absence of extensive cell wall substance facilitates the diffusion of

molecules from one cell into the other. An easier way for molecules to move between plant cells is by means of the *plasmodesmata* (singular; *plasmodesma*). These are fine, membrane-flanked, cytoplasmic threads that pass from a protoplast, through a pit, directly into the protoplast of a second cell (see Ledbetter and Porter or O'Brien and McCully). A tobacco leaf cell can have 10 to 30 plasmodesmata per $100 \mu\text{m}^2$ of cell surface, each plasmodesma having a diameter near $0.2 \mu\text{m}$. Plasmodesmata can be as small as 20 nm in diameter and their frequency in the hundreds per $100 \mu\text{m}^2$. Usually they occupy about 0.1 to 0.5% of the surface area of the cell. The continuum of communicating cytoplasm created by such intercellular connections is commonly referred to as the *symplasm*. The presence of the plasmodesmata provides a particularly effective pathway for solute movement between adjacent cells since no permeability barrier in the form of either a cell wall or a membrane needs to be surmounted for diffusion of molecules from cell to cell in the symplasm (see also Tyree).

Since movement from cell to cell through the symplasm is physiologically so important, let us next compare it with the concomitant diffusion across the plasmalemma. We will use Fick's first law as presented in Equation 1.8, viz., $J_j = D_j K_j \Delta c_j / \Delta x = P_j \Delta c_j$. Let us consider two adjacent cells in which the glucose concentration differs by 1 mM. Glucose is rather insoluble in membrane lipids and would have a permeability coefficient of about 10^{-7} cm/sec for a typical plasmalemma. For movement from cell to cell across the two plasmalemmas in series, the effective P_j would be 0.5×10^{-7} cm/sec (inclusion of the cell wall as another series barrier for diffusion would only slightly reduce the effective P_j in this case—also, see Problem 1.5 for handling barriers in series). Thus, the flux of glucose across the plasmalemmas toward the cell with the lower concentration would be $(0.5 \times 10^{-7} \text{ cm/sec})(1 \times 10^{-3} \text{ mole}/1000 \text{ cm}^3)$, or $5 \times 10^{-14} \text{ mole}/\text{cm}^2\text{-sec}$. Let us next consider the flux for the symplasm. In the pores of the plasmodesmata, D_{glucose} should be similar to its value in water, $0.7 \times 10^{-5} \text{ cm}^2/\text{sec}$ (see p. 17), while K_{glucose} would be 1.0. We will let the plasmodesmata be $2 \mu\text{m}$ long. The flux in the pores would thus be $(0.7 \times 10^{-5} \text{ cm}^2/\text{sec})(1.0)(1 \times 10^{-3} \text{ mole}/1000 \text{ cm}^3)/(2 \times 10^{-4} \text{ cm})$, or $4 \times 10^{-8} \text{ mole}/\text{cm}^2\text{-sec}$. If the pores occupy 0.002 of the surface area of the cells, then the rate of glucose diffusion through the plasmodesmata per unit area of the cells would be $(0.002)(4 \times 10^{-8})$, or $8 \times 10^{-11} \text{ mole}/\text{cm}^2\text{-sec}$. This is more than a thousandfold greater than the simultaneously occurring diffusion across the plasmalemmas. The permeability coefficients of the plasmalemmas for phosphorylated sugars like ribose-5-phosphate or ribulose-1,5-diphosphate would be less than for glucose, while the diffusion coefficients in the plasmodesmata would be about

the same as for glucose. Thus, the discrepancy between fluxes of charged species through the plasmodesmata and those across the plasmalemmas would be even greater than the thousandfold difference calculated here for glucose. The joining of cytoplasms into a symplasm indeed facilitates the diffusion of solutes from one cell to another.

Stress-strain relations of cell wall. Cell walls of mature plant cells are generally quite resistant to mechanical stretching, especially when there is appreciable thickening of the secondary cell walls. Nevertheless, such structures will stretch when a stress is applied. Reversible elastic properties are generally described in terms of a measure of elasticity known as *Young's modulus*, which is the ratio of applied stress (force per unit area) to the resulting strain (fractional change in length):

$$\text{Young's modulus} = \frac{\text{stress}}{\text{strain}} = \frac{\text{force/area}}{\Delta L/L} \quad (1.13)$$

Since $\Delta L/L$ is dimensionless, Young's modulus has the dimensions of force per unit area or pressure. A high value of this modulus of elasticity means that a large stress must be applied in order to produce a noticeable effect.

Young's modulus for dry cotton fibers (which represent nearly pure cellulose) is quite large, about 10^5 kilograms/cm², or 5% of that for steel (see Siegel). Because of both the complicated three-dimensional array of microfibrils in the cell wall and the presence of many other components, Young's modulus for a cell wall is considerably less than for pure cellulose. The modulus of elasticity for the cell wall of *Nitella*, for example, has been experimentally found to be about 7000 kg/cm² (see Kamiya *et al.*). We will use this latter value when we indicate the fractional stretching that can occur for plant cells.*

The hydrostatic (turgor) pressure, P , acts uniformly in all directions in a cell. This internal pressure tends to cause the cell to expand and also leads to tensions (stresses) in the cell wall. The magnitude of these stresses varies with the physiological state of the plant as well as with direction in the cell wall, an aspect that we will consider next.

A cylinder, closely approximating the large internodal cells of *Nitella* (Fig. 3.4) or *Chara*, is a useful geometrical model for evaluating the various cell wall stresses. Let the radius of the cylinder be r (as in Fig. 1.12). The force on an end wall is the pressure (P) times the area of the end wall, πr^2 . The

* See the footnote to Appendix III for an explanation of the common convention used here to express forces, such as those in Young's modulus (Eq. 1.13), in units of mass, e.g., kilograms in the present case.

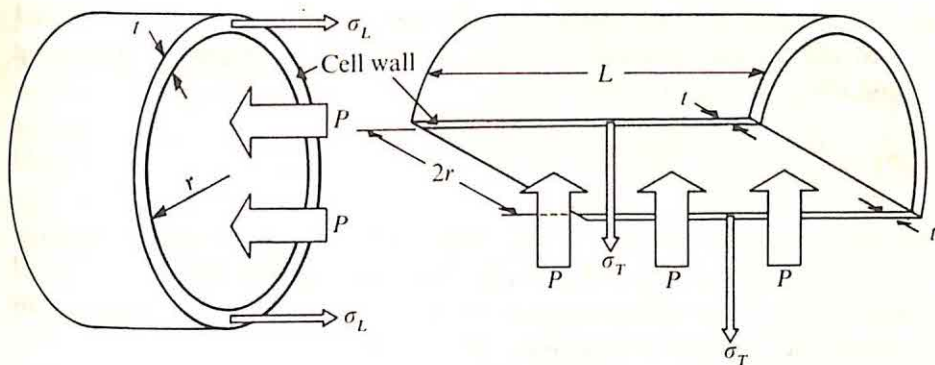


Figure 1.12

Schematic sections of a hypothetical cylindrical cell resembling the internodal cells of *Nitella* or *Chara*, illustrating various dimensions and the stresses (σ_L and σ_T) existing in the cell wall. One way to calculate the stresses is to imagine that the cellular contents are removed, leaving only the cell wall, which has a uniform hydrostatic pressure P acting perpendicular to its inside surface. The projection of this P over the appropriate area gives the force acting in a certain direction, while the reaction to this force is an equal force in the opposite direction in the cell wall. By dividing the force in the cell wall by the area over which it occurs, we can determine the cell wall stress.

force is then $P\pi r^2$. This force is balanced by a tension known as the *longitudinal stress*, σ_L , which occurs in the cell wall. The area over which σ_L acts is shown by the cut portion of the cell wall in the left part of Figure 1.12. The longitudinal stress occurs in an annulus of circumference $2\pi r$ (approximately) and a width equal to the thickness of the cell wall, t . The force in the cell wall is thus $(\sigma_L)(2\pi r)(t)$. This force is an equal and opposite reaction to $P\pi r^2$, and so $\sigma_L 2\pi r t$ equals $P\pi r^2$ or

$$\sigma_L = \frac{rP}{2t} \quad (1.14)$$

The longitudinal stress acts parallel to the major axis of the cylinder and resists the lengthwise deformation of the cell.

A *tangential stress*, σ_T , also exists in the cell wall in response to the internal pressure and limits the radial expansion of the cell. In order to determine the magnitude of this stress, we will consider a cell split into half along its axis (right side of Fig. 1.12). The split part of the cell has an area of $2rL$ in the plane of the cut, which is acted on by the pressure P , leading to a force of $P2rL$. This force is resisted by the tangential stress in the cell wall. As shown in Figure 1.12, σ_T acts along two cell wall surfaces each of width t and length L . The total force in the cut part of the cell wall is thus the

area ($2tL$) times the tangential stress. Equating this force ($\sigma_T 2tL$) with that due to the internal pressure in the cell ($P2rL$), we obtain the following relationship for the tangential stress:

$$\sigma_T = \frac{rP}{t} \quad (1.15)$$

The tangential stress in the cell wall given by Equation 1.15 is twice as large as the longitudinal stress (Eq. 1.14). Thus, this simple cylindrical model illustrates the important point that the stresses in a cell wall can vary with direction (see Lockhart in Bonner and Varner).

To estimate the magnitudes of the stresses and the resulting strains in the cell wall, let us consider a *Nitella* or *Chara* cell that is 1 mm in diameter with a cell wall $5 \mu\text{m}$ thick. In this case, r/t is $(500 \mu\text{m})/(5 \mu\text{m})$, or 100. A reasonable estimate of P is 6 bars. (A bar is a convenient unit for expressing pressures occurring in plants and equals 10^6 dynes/cm² or 0.987 atmosphere—see App. III.) Using Equation 1.14, we can calculate that the longitudinal stress in the cell wall of such a cell is $[(100)(6 \text{ bars})]/(2)$, or 300 bars. By Equation 1.15, the tangential stress would be twice as great, or 600 bars. One bar corresponds to 1.020 kg/cm² at sea level (App. III). The longitudinal stress in the cell wall is thus $(300 \text{ bars})(1.020 \text{ kg/cm}^2\text{-bar})$, or 306 kg/cm², an appreciable tension. The strain produced by this stress can be calculated using the definition of Young's modulus (Eq. 1.13) and its particular value for the cell wall of *Nitella*, 7000 kg/cm². From Equation 1.13, the fractional change in length is then $(306 \text{ kg/cm}^2)/(7000 \text{ kg/cm}^2)$, or 0.044, i.e., only about 4% in this case. Hence, even with an internal pressure of 6 bars, which leads to a longitudinal stress in the cell wall of 300 bars, the cell wall (and consequently the whole cell) is not extended very much. The cell wall is indeed rigid and therefore well-suited for limiting the size of individual cells as well as for providing the structural support of a plant.

Before concluding this discussion of cell walls, it should be noted that the case of elasticity or reversible deformability is only one extreme of stress-strain behavior. At the opposite extreme is plastic (irreversible) extension. If the amount of strain is directly proportional to the time that a certain stress is applied, and if it persists when the stress is removed, we have an example of viscous flow. The cell wall actually exhibits intermediate properties and is said to be *viscoelastic*. When a given stress is applied to a viscoelastic material, it turns out that the resulting strain is approximately proportional to the logarithm of time. Moreover, such extension is partly elastic (reversible) and partly plastic (irreversible). Underlying the viscoelastic behavior of the cell wall are the crosslinks between the various

polymers. For example, if a bond from one cellulose polymer to another is broken while the cell wall is under tension, the opportunity exists for a new bond to form in a less strained configuration, leading to an irreversible or plastic extension of the cell wall. The quantity responsible for the tension in the cell wall—which in turn leads to such viscoelastic extension—is the hydrostatic pressure within the cell (see Cleland).

Problems

1.1 A cylindrical *Nitella* cell is 10 cm long and 1 mm in diameter, a spherical *Valonia* cell 1 cm in diameter, and a spherical *Chlorella* cell 4 μm in diameter. (a) What is the area/volume in each case? (b) Which cell has the largest amount of surface area per unit volume? (c) If it takes 1 sec for the internal concentration of ethanol, which is initially absent from the cells, to reach half the external concentration for *Chlorella*, how long would it take for *Nitella* and *Valonia*? Assume that P_{ethanol} is the same for all the cells. (d) Assume that the cell walls are equal in thickness. For a given internal pressure, which cell would have the highest cell wall stress?

1.2 A thin layer of some solution is inserted into a long column of water. One hour later, the concentration of the solute is 0.1 molal at the plane of insertion and 0.037 molal at a distance 3 mm away. (a) What is its diffusion coefficient? (b) When the concentration 9 cm away is 37% of the value at the plane of insertion, how much time has elapsed? (c) How many moles of solute per unit area were initially inserted into the column of water? (d) Suppose that a trace amount of a substance having a diffusion coefficient 100 times smaller than that of the main solute was also initially introduced. For the time in (b), where would its concentration drop to $1/e$ of the value at the plane of insertion?

1.3 Let us suppose that mitochondria with a volume of $0.30 \mu\text{m}^3$ each and a density of 1.10 g/cm^3 diffuse like a chemical species. (a) What is the “molecular weight” of mitochondria? (b) Suppose that a chemically similar species of molecular weight 200 g/mole has a diffusion coefficient of $0.5 \times 10^{-5} \text{ cm}^2/\text{sec}$. If diffusion coefficients are inversely proportional to the cube root of molecular weights for this series of similar species, what is $D_{\text{mitochondria}}$? (c) If we assume that Equation 1.6 can adequately describe such motion, how long would it take on the average for a mitochondrion to diffuse $0.2 \mu\text{m}$ (a distance just discernible using a light microscope)? How long would it take for the mitochondrion to diffuse $50 \mu\text{m}$ (the distance across a typical leaf cell)? (d) If D_{ATP} is $0.3 \times 10^{-5} \text{ cm}^2/\text{sec}$, how long would it take ATP to diffuse $50 \mu\text{m}$? Relate your answer to cellular use of the ATP produced by respiration—see part (c) of this problem.

1.4 Suppose that an unstirred air layer 1 mm wide is adjacent to a guard cell with a cell wall $2 \mu\text{m}$ thick. (a) Assume that an (infinitely) thin layer of $^{14}\text{CO}_2$ is intro-

duced at the surface of the guard cell. If D_{CO_2} is 10^6 times larger in air compared with the cell wall, what are the relative times for $^{14}\text{CO}_2$ to diffuse across the two barriers? (b) If it takes $^{14}\text{CO}_2$ just as long to cross an 8 nm plasmalemma as it does to cross the cell wall, what are the relative sizes of the two diffusion coefficients (assume that the $^{14}\text{CO}_2$ was introduced in a plane between the two barriers)? (c) Assuming that the partition coefficient for CO_2 is 100 times greater in the cell wall than in the plasmalemma, in which barrier is the permeability coefficient larger and by how much?

1.5 Without correcting for the effect of an unstirred layer 20 μm thick outside a membrane 7.5 nm in thickness, the apparent or total permeability coefficients were measured to be 1.0×10^{-2} cm/sec for D_2O , 2.0×10^{-3} cm/sec for methanol, and 3.0×10^{-6} cm/sec for L-leucine. For barriers in series, the overall permeability coefficient for species j (P_j^{total}) is related to those of the individual barriers (P_j^i) as follows: $1/P_j^{\text{total}} = \sum_i 1/P_j^i$. For purposes of calculation, we will assume that

in the present case the unstirred layer on the inner side of the membrane is negligibly thin. (a) What is P_j for the external unstirred layer for each of the compounds? Assume that $D_{\text{D}_2\text{O}}$ is equal to 2.6×10^{-5} cm²/sec, D_{methanol} is 0.8×10^{-5} cm²/sec, and D_{leucine} equals 0.2×10^{-5} cm²/sec in water at 25°. (b) What are the permeability coefficients of the three compounds for the membrane? (c) From the results in (a) and (b), what are the main resistances for the diffusion of the three compounds in this case? (d) What are the highest possible values at 25° of P_j^{total} for each of the three compounds moving across an unstirred layer of 20 μm and a membrane in series?

1.6 Consider a solute having a permeability coefficient of 10^{-4} cm/sec for the plasmalemma of a cylindrical *Chara* cell that is 10 cm long and 1 mm in diameter. Assume that its concentration remains essentially uniform within the cell. (a) How much time would it take for 90% of the solute to diffuse out into a large external solution initially devoid of that substance? (b) How much time would it take if diffusion occurred only at the two ends of the cell? (c) How would the times calculated in (a) and (b) change in order that 99% of the solute would diffuse out? (d) How would the times change if P_j were 10^{-6} cm/sec?

References

- Bonner, J., and J. E. Varner (eds.). 1965. *Plant Biochemistry*, Academic Press, New York.
- Bull, H. B. 1964. *An Introduction to Physical Biochemistry*, F. A. Davis, Philadelphia.
- Cleland, R. 1971. Cell wall extension. *Annual Review of Plant Physiology* 22: 197-222.
- Clowes, F. A. L., and B. E. Juniper. 1968. *Plant Cells*, Blackwell Scientific Publications, Oxford.

- Crank, J. 1956. *The Mathematics of Diffusion*, Oxford University Press, Oxford.
- Dainty, J. 1963. Water relations of plant cells. *Advances in Botanical Research* 1: 279-326.
- Davson, H., and J. F. Danielli. 1952. *The Permeability of Natural Membranes*, 2nd ed., Cambridge University Press, Cambridge.
- Diamond, J. M., and E. M. Wright. 1969. Biological membranes: The physical basis of ion and nonelectrolyte selectivity. *Annual Review of Physiology* 31: 581-646.
- Douce, R., R. B. Holtz, and A. A. Benson. 1973. Isolation and properties of the envelope of spinach chloroplasts. *Journal of Biological Chemistry* 248: in press.
- Epstein, E. 1973. Roots. *Scientific American* 228: No. 5 (May), 48-58.
- Esau, K. 1965. *Plant Anatomy*, 2nd ed., Wiley, New York.
- Green, D. E., and H. Baum. 1970. *Energy and the Mitochondrion*, Academic Press, New York.
- Green, K., and T. Otori. 1970. Direct measurements of membrane unstirred layers. *Journal of Physiology* 207: 93-102.
- Jacobs, M. H. 1967. *Diffusion Processes*, Springer Verlag, New York.
- Kamiya, N., M. Tazawa, and T. Takata. 1963. The relation of turgor pressure to cell volume in *Nitella* with special reference to mechanical properties of the cell wall. *Protoplasma* 57: 501-521.
- Ledbetter, M. C., and K. R. Porter. 1970. *Introduction to the Fine Structure of Plant Cells*, Springer Verlag, New York.
- Northcote, D. H. 1972. Chemistry of the plant cell wall. *Annual Review of Plant Physiology* 23: 113-132.
- O'Brien, T. P., and M. E. McCully. 1969. *Plant Structure and Development*, Macmillan, New York.
- Park, R. B., and P. V. Sane. 1971. Distribution of function and structure in chloroplast lamellae. *Annual Review of Plant Physiology* 22: 395-430.
- Rothfield, L. I. (ed.). 1971. *Structure and Function of Biological Membranes*, Academic Press, New York.
- Sarkanen, K. V., and C. H. Ludwig (eds.). 1971. *Lignins: Occurrence, Formation, Structure and Reactions*, Interscience (Wiley), New York.
- Siegel, S. M. 1962. *The Plant Cell Wall*, Pergamon Press, New York.
- Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science* 175: 720-731.
- Sober, H. A. (ed.). 1970. *Handbook of Biochemistry with Selected Data for Molecular Biology*, 2nd ed., Chemical Rubber, Cleveland.
- Stein, W. D. 1967. *The Movement of Molecules Across Cell Membranes*, Academic Press, New York.
- Tanford, C. 1961. *Physical Chemistry of Macromolecules*, Wiley, New York.
- Tyree, M. T. 1970. The symplast concept. *Journal of Theoretical Biology* 26: 181-214.
- Weast, R. C. (ed.). 1973. *Handbook of Chemistry and Physics*, 54th ed., Chemical Rubber, Cleveland.
- Wright, E. M., and J. M. Diamond. 1969. Patterns of non-electrolyte permeability. *Proceedings of the Royal Society, Series B*, 172: 227-271.

CHAPTER TWO

WATER

Water is the main constituent of most plant cells, as our discussion of both vacuoles and cell walls in the previous chapter has already suggested. The actual cellular water content varies with cell type and physiological condition. For example, the carrot root is about 85% water by weight, while the young inner leaves of lettuce contain up to 95% water. Water comprises only 10% of certain dry seeds and spores, but when these become metabolically active, an increase in water content is essential for the transformation.

Water is (1) the medium in which diffusion of solutes takes place in plant cells (2) an extremely suitable substance for temperature regulation (3) the solvent for many biochemical reactions, and (4) rather incompressible, the latter property underlying its role in plant support. In thermal properties, water has a high heat of vaporization, a high heat capacity, and a high thermal conductivity for a liquid, all of which make it ideal for maintaining temperature uniformity. Water is also an extremely good general solvent, in part owing to its small-sized molecules. Because of its polar character, water is suitable for dissolving polar substances. Its high dielectric constant makes it a particularly appropriate solvent for ions. This latter point has far-reaching consequences for life, since most biologically important solutes are electrically charged. The mineral nutrients needed for growth and the organic products of photosynthesis are transported throughout the plant in aqueous solutions. In actively growing land plants, water continuity exists from the soil, through the plant, to the evaporation sites in the leaves. Water is quite transparent to visible radiation, enabling sunlight to reach chloroplasts within the cells in leaves and to plants submerged at appreciable depths in lakes and oceans. Water is also intrinsically involved with metabolism. It is the source of O_2 evolved in photosynthesis and the hydrogen used for CO_2 reduction. The generation of the important energy currency, ATP,

involves the extraction of the components of water from ADP plus phosphate, i.e., such a phosphorylation is a *dehydration* process which takes place in an *aqueous* solution under biological conditions. Thus, a recognition of the many unique properties of water is crucial for an overall understanding of physiology.

A number of isotopically different forms of water can be prepared, which greatly facilitates experimental studies with this biologically important molecule. If deuterium (^2H) replaces both of the usual hydrogens, the result is "heavy water," or deuterium oxide with a molecular weight of 20. The entry of water into chemical reactions in the plant can then be studied by analyzing the deuterium content of the various substances involved as reactants or products. Also, tritium (^3H), which is radioactive and has a half-life of 12.4 years, can be incorporated into water. Such tritiated water has been invaluable in measuring the rate of diffusion of water in plant tissue. As another alternative for tracing the pathway of water, the usual ^{16}O isotope can be replaced by ^{18}O . This "labeling" of water with ^{18}O was used to determine that the oxygen evolved in photosynthesis actually comes from water and not from carbon dioxide (see Ch. 5).

Physical properties

Water differs markedly from substances having closely related electronic structures, a point which we can illustrate by considering the series CH_4 , NH_3 , H_2O , HF , and Ne . Each member contains 10 protons and 10 electrons, but the number of hydrogen atoms decreases along the series from methane to neon. For this sequence, the melting points are -184° , -78° , 0° , -92° , and -249° , respectively. A similar striking variation in boiling points occurs, viz., -161° , -33° , 100° , 19° , and -246° . The relatively high melting and boiling points for water, compared with substances having a similar electronic structure, are indicative of its strong intermolecular forces. In other words, thermal agitation does not easily disrupt the water-water bonding. This attraction between molecules is responsible for many characteristic features of water.

Hydrogen bonding. The strong intermolecular forces in water result from the structure of the H_2O molecule (Fig. 2.1). The internuclear distances between the oxygen and each of the two hydrogens are approximately $0.99 \text{ \AA}$, and the $\text{H}-\text{O}-\text{H}$ angle is about 105° . The oxygen atom is strongly electronegative and tends to draw electrons away from the hydrogen atoms. This leaves the oxygen with a partial negative charge (δ^- in Fig. 2.1), while

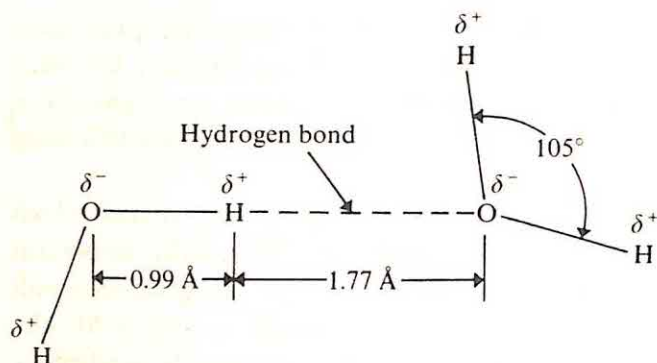


Figure 2.1

Schematic structure of water molecules, indicating the hydrogen bonding resulting from the electrostatic attraction between the net positive charge on a hydrogen (δ^+) in one molecule and the net negative charge on an oxygen (δ^-) in a neighboring water molecule.

the two hydrogens become positively charged (δ^+). The positively charged hydrogens of a water molecule are electrostatically attracted to the negatively charged oxygens in two neighboring water molecules. This leads to "hydrogen bonding" between water molecules, with an energy of about 4.8 kilocalories (kcal)/mole of hydrogen bonds. Such bonding of water molecules to each other leads to increased order in aqueous solutions. In fact, liquid water becomes nearly crystalline in local regions, a condition which is extremely important for determining the molecular interactions and orientations that occur in aqueous solutions.

Ice is a coordinated crystalline structure in which essentially all the water molecules are joined by hydrogen bonds. When heat is added so that the ice melts, some of these intermolecular hydrogen bonds are broken. The heat of fusion of ice at 0° is 80 cal/g or 1.44 kcal/mole. The total rupture of the intermolecular hydrogen bonds involving each of its hydrogens would require 9.6 kcal/mole of water.* The heat of fusion thus indicates that $(100)(1.44)/(9.6)$, or at most 15% of the hydrogen bonds are broken when ice melts (some energy is needed to overcome van der Waals attractions, so that less than 15% of the hydrogen bonds are actually broken upon

*The actual magnitude of the hydrogen bond energy assigned to ice depends somewhat on the particular operational definition used in the measurement of the various bonding energies. Therefore, the quoted values vary somewhat. See Eisenberg and Kauzmann as well as Pauling.

melting). Conversely, over 85% of the hydrogen bonds remain intact for liquid water at 0°. Since 1.00 cal is needed to heat 1 g of water 1°, (1.00 cal/g-°)(25°)(18 g/mole) ($\frac{1}{1000}$ kcal/cal), or 0.45 kcal/mole is required to heat water from 0° to 25°. If all of this energy were used to break hydrogen bonds, over 80% of the bonds would still remain intact at 25°. Such bonding leads to the semicrystalline order found in aqueous solutions. The extensive amount of intermolecular hydrogen bonds present in the liquid state contributes to the unique and biologically important properties of water that we will discuss throughout this chapter.

The energy required to separate molecules from a liquid and move them into an adjacent vapor phase without a change of temperature is called the *heat of vaporization*. For water, the heat of vaporization at 100° is 539 cal/g or 9.71 kcal/mole. Per gram, this is the highest heat of vaporization of any known liquid, and it reflects the large amount of energy required to disrupt the extensive hydrogen bonding in aqueous solutions. More pertinent to plant physiology is the heat of vaporization of water at temperatures encountered by plants. At 25° each mole of water evaporated takes 10.5 kcal (see App. II), which means that a substantial heat loss by the plant accompanies the evaporation of water in transpiration. (This will be discussed in Ch. 7.) Most of this vaporization energy is needed to break hydrogen bonds so that the water molecules can become separated in the gaseous phase. For example, if 80% of the hydrogen bonds remained at 25°, then (0.80)(9.6), or 7.7 kcal/mole would be needed just to rupture them—other energy is needed to overcome the attractive van der Waals forces and for the expansion in going from a liquid to a gas. The heat loss upon evaporation of water is one of the primary means of temperature regulation in land plants. It dissipates much of the energy gain resulting from the net radiation balance. (Other important heat losses occur by conduction and convection—see Ch. 7.)

Surface tension. Water is further characterized by having an extremely high surface tension, which is evident at an interface between water and air. Surface tension can be defined as the force transmitted across a line in the surface, and its value at an air-water interface is 72.8 dynes/cm at 20°. (See App. II for values at other temperatures.) Perhaps a better way of defining surface tension is to regard it as the amount of energy required to expand the surface by unit area. (Since a dyne is an erg/cm, note that surface tension has not only the dimensions of force per unit length but also the units of ergs/cm², or energy per unit area.) To see why energy is required to expand the water surface, let us consider water molecules which are brought from the interior of an aqueous phase to an air-water interface.

If this involves only an expansion of the surface area—i.e., if there is no accompanying movement of other water molecules from the surface to the interior—then a loss in the water-water attraction of intermolecular hydrogen bonds occurs with no compensating air-water attraction. Energy (72.8 ergs to increase the area by 1 cm^2) is thus needed to break the hydrogen bonds involved in moving water molecules from the interior of the solution to the air-water interface. In fact, the term “surface free energy” is more appropriate from a thermodynamic point of view than is the conventional term “surface tension.”

The surface tension of an aqueous solution is ordinarily only slightly influenced by the composition of the adjacent gas phase, but it can be markedly affected by certain solutes. Molecules are far apart in a gas—the density of dry air at 0° and 760 mm Hg is only 1.293 mg/cm^3 —so that the frequency of collisions or other interactions between molecules in the gas phase and those in the liquid phase is relatively low. Certain solutes, such as sucrose or KCl, do not preferentially collect at the air-liquid interface and consequently have little effect on the surface tension of an aqueous solution. On the other hand, fatty acids and certain lipids may become concentrated at interfaces and greatly reduce surface tensions. For example, 10 mM caproic acid (a 6-carbon fatty acid) lowers the surface tension about 15 dynes/cm below the value for pure water, while only 0.05 mM capric acid (a 10-C fatty acid) lowers it by about 25 dynes/cm (see Bull). Substances such as soaps (salts of fatty acids) or denatured proteins with large hydrophobic side chains may collect nearly exclusively at the interface and reduce the surface tension of aqueous solutions to as low as 20 dynes/cm. A common feature of such “surfactant” molecules is that they have both polar and nonpolar regions.

Capillary rise. The intermolecular attraction occurring between like molecules in the liquid state, such as the water-water attraction based on hydrogen bonds, is referred to as *cohesion*. The attractive interaction between a liquid and a solid phase, such as between water and the walls of a small tube or capillary, is called *adhesion*. When the water-wall attraction is appreciable compared with the water-water cohesion, the walls are said to be *wettable*, and water then rises in the capillary. At the opposite extreme, where the intermolecular cohesive forces within the liquid are substantially greater than the adhesion between the liquid and the wall material, the upper level of the liquid in a capillary is lower than the surface of the free solution. Such capillary depression occurs for liquid mercury in glass capillaries. For water in glass capillaries or in xylem vessels, the attraction between the water molecules and the walls is great, therefore the liquid rises.

Since capillary rise has important implications in plant physiology, we will discuss its characteristics in a quantitative manner.

As an example appropriate to the evaluation of water rise in a plant, let us consider a wettable capillary of inner radius r dipping into some aqueous solution (Fig. 2.2). The strong adhesion of water molecules to the wettable wall leads to a rise of the fluid in the capillary. Since there is also a strong water-water cohesion in the bulk solution, water is concomitantly pulled up into the lumen of the capillary as water rises along the walls. Viewed another way, the air-water surface greatly resists being stretched, a property reflected in the high surface tension of water at gas-liquid interfaces. This resistance tends to minimize the area of the air-water interface, a condition achieved if water also moves up in the lumen as well as along the sides of the capillary. The tendency for water to rise along the walls of the capillary is thus transmitted to a volume of fluid. Let the height that the liquid rises in the capillary be h and the contact angle that the liquid makes with the capillary wall be α (Fig. 2.2). The extent of the rise depends on α , so the properties of the contact angle will now be examined a little more closely.

The size of the contact angle depends on the magnitude of the liquid-solid adhesive force compared with that of the liquid-liquid cohesion. Specifically, Young's equation indicates that the adhesion equals $(1 + \cos \alpha)/2$ times the cohesion. (Sometimes this relation is called the Young and Dupre equation—see Adamson, Bikerman, and/or Davies and Rideal for an authoritative treatment.) Consequently, when the adhesive force equals (or exceeds)

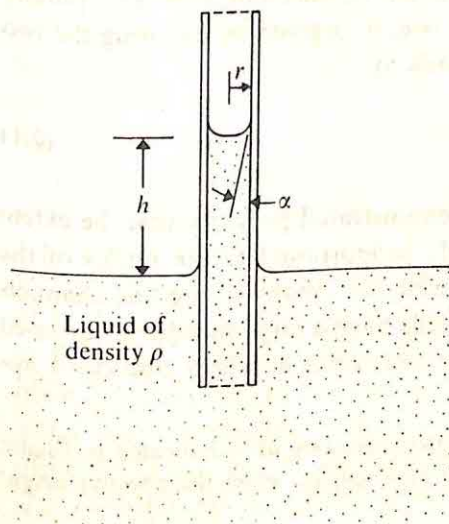


Figure 2.2

Quantities involved in the capillary rise of a liquid.

the cohesive force in the liquid, $\cos \alpha$ must be unity, therefore the contact angle α equals zero ($\cos 0^\circ = 1$). This is the case for water in capillaries made of clear smooth glass or having walls with polar groups on the exposed surface. When the adhesive force equals half of the value of the cohesive force, $\cos \alpha$ is then zero and the contact angle is then 90° ($\cos 90^\circ = 0$). In this case, the level of the fluid in the capillary is the same as that in the bulk of the solution. This latter condition is closely approached in water-polyethylene adhesion, where α equals 94° . As the liquid-solid adhesive force becomes relatively less compared with the intermolecular cohesion in the liquid phase, the contact angle (indicated in Fig. 2.2) increases toward 180° and capillary depression occurs, e.g., water has an α of about 110° for paraffin, while the contact angle for mercury interacting with a glass surface is near 150° .

We can calculate the extent of the capillary rise by considering the balance of two forces: (1) gravity acting downward and (2) surface tension, which leads to a force in the upward direction in the case of wettable walls in a vertical tube (cf. Fig. 2.2). The force pulling upward acts along the inside perimeter of the capillary, a distance of $2\pi r$, with a force per unit length equal to σ , the surface tension. However, the component of the force actually acting vertically upward is $2\pi r \sigma \cos \alpha$ (where α is the contact angle illustrated in Fig. 2.2). This upward force is balanced by the gravitational force acting on the liquid of density ρ and volume essentially $\pi r^2 h$.* The gravitational force is simply the mass involved times the gravitational acceleration g ($F = ma$, Newton's second law of motion). In the present case gravity acts on a mass of fluid of approximately $\pi r^2 h \rho$, so the gravitational force is $\pi r^2 h \rho g$ acting downward. Since this force is balanced by $2\pi r \sigma \cos \alpha$ pulling in the opposite direction, the extent of rise, h , is given by equating the two forces ($\pi r^2 h \rho g = 2\pi r \sigma \cos \alpha$), which leads to

$$h = \frac{2\sigma \cos \alpha}{r\rho g} \quad (2.1)$$

Equation 2.1 indicates the readily demonstrated property that the extent of liquid rise in a capillary is inversely proportional to the radius of the tube. For water in glass capillaries as well as in many of the fine channels encountered in plants, where the cell walls have a large number of exposed polar groups, the contact angle can be near zero, in which case $\cos \alpha$ ap-

* Some fluid is also held in the "rim" of the meniscus, as is indicated in Figure 2.2. For narrow capillaries, the fluid in the meniscus increases the effective height of the column by about $r/3$.

pearing in Equation 2.1 can be set equal to 1. The density of water is 0.998 g/cm^3 at 20° (actually, ρ in Eq. 2.1 is the difference between the liquid density and that of the displaced air, the latter being about 0.001 g/cm^3), and the acceleration due to gravity, g , is about 980 cm/sec^2 (see App. II). Using a surface tension for water at 20° of 72.8 dynes/cm (App. II), we obtain the following relation between the height of the rise and the capillary radius:

$$h_{(\text{cm})} = \frac{0.149 \text{ cm}^2}{r_{(\text{cm})}}$$

or

$$h_{(\text{m})} = \frac{1.49 \times 10^{-5} \text{ m}^2}{r_{(\text{m})}} \quad (2.2)$$

where the subscripts (cm) or (m) in Equation 2.2 mean that the dimensions involved are expressed in centimeters or meters, respectively.

Capillary rise in the xylem. Although Equations 2.1 and 2.2 refer only to the height of capillary rise in a static sense, they still have important implications concerning the movement of water in plants. To be specific, let us consider a xylem vessel having a radius of $20 \mu\text{m}$. From Equation 2.2, water will rise in it to a height of $(1.49 \times 10^{-5} \text{ m}^2)/(20 \times 10^{-6} \text{ m})$, or 0.75 m . This capillary rise would be sufficient to account for the extent of the upward movement of water in small plants, although it says nothing about the rate of such movement (which we will consider in Ch. 8). For water to reach the top of a 30 meter tree by capillary action, however, the vessel would have to be $(1.49 \times 10^{-5} \text{ m}^2)/(30 \text{ m})$ or $5 \times 10^{-7} \text{ m}$, which is $0.5 \mu\text{m}$ in radius. This is much smaller than that observed for xylem vessels, indicating that capillary rise in channels of the size of the xylem cells cannot account for the extent of the water rise in tall trees. Furthermore, the vascular system is not open to the air at the upper end, and thus xylem vessels are not really analogous to the capillary depicted in Figure 2.2.

The numerous interstices in the cell wall of xylem vessels form a mesh-work of many small tortuous capillaries, which can lead to an extensive capillary rise of water in a tree. A representative "radius" for these channels in the cell wall might be 5 nm ($50 \text{ \AA}$). Based on Equation 2.2, a capillary of 5 nm radius could support a water column of $(1.49 \times 10^{-5})/(5 \times 10^{-9})$, or $3 \times 10^3 \text{ m}$ —which is $3 \text{ kilometers (km)}$ —far in excess of the needs of any plant. In other words, the cell wall could act as a very effective wick for water rise in its numerous small interstices, although the actual rate of such movement up a tree would generally be far from sufficient to replace the water lost by transpiration (see Ch. 8).

Because of the appreciable water-wall attraction which can develop both at the top of a xylem vessel and in the numerous interstices of its cell walls, water that is already present in the lumen of a xylem vessel can be sustained or supported at great heights. The upward force, transmitted to the rest of the solution in the xylem vessel by water-water cohesion, overcomes the gravitational pull downward. Thus, the key feature for holding up water that is already present in the xylem vessel against the pull of gravity is the very potent attractive interaction (adhesion) of water to the cell wall surfaces at the top of the vessel. Two aspects of this warrant further consideration. First, what happens if the lumen of the xylem vessel becomes filled with air? Will water then refill it? The capillary rise of water is not sufficient to refill most xylem vessels greater than about 1 m in length, so that, in general, air-filled vessels are lost for conduction, such as the loss which occurs in the inner annual rings of most trees. The other aspect is more subtle, and we will consider it a little more extensively.

Tensile strength. The pulling on water columns which occurs in capillary rise and in the sustaining of water in the xylem requires that water be put under tension or negative pressure. In other words, water must have a definite tensile strength, the maximum tension (force per unit area) that it can withstand before breaking. In fact, the intermolecular hydrogen bonds resist the pulling apart of water in a column. If 80% of the hydrogen bonds are intact in water at 25°, their energy will be $(0.80)(9.6)$ or 7.7 kcal/mole, which is $(7.7 \text{ kcal/mole})/(18 \text{ g/mole})$ or 0.43 kcal/g of water. For a density of 1.00 g/cm^3 , and replacing kcal by 4.184×10^{10} ergs (App. III), we calculate that the energy of the hydrogen bonds is 1.8×10^{10} ergs/cm³. The tension that is applied to a water column acts against this attractive energy of the hydrogen bonds. When the fracture is just about to occur at each hydrogen bond, the maximum possible tensile strength is developed. Thus, the maximum tensile strength would represent an input of energy of 1.8×10^{10} ergs/cm³. Since an erg is a dyne-cm and a bar is 10^6 dynes/cm² (App. III), the maximum tensile strength of water corresponds to 18,000 bars. We might also attempt to calculate the maximum tensile strength of water by considering the electrostatic force of attraction between the δ^+ on the hydrogen of one water molecule and the δ^- on the oxygen of a neighboring one (cf. the hydrogen bond in Fig. 2.1). Dividing this force by the area of the water molecules involved, we could then calculate the tension developed—but such an approach involves formidable geometrical considerations.

Experimentally, water withstands negative pressures (tensions) only up to approximately 300 bars at 20° without breaking. (Observed tensile strengths depend on the wall material, the diameter of the vessel in which the determinations are made, and any solutes present in the water—see

Hayward.) Any local imperfections in the semicrystalline structure of water—such as those caused by H^+ and OH^- , which are always present, even in pure water—markedly reduce the observed tensile strength from the maximum value predicted based on hydrogen bond strengths. Nevertheless, the measurable tensile strength for water provided by the intermolecular hydrogen bonds (up to 300 bars) is nearly 10% of that for copper or aluminum, and is sufficiently high to meet the demands encountered for water movement in plants. In summary, the great cohesive forces between water molecules allow an appreciable tension to exist in an uninterrupted water column in a wettable capillary or tube such as a xylem vessel. This property is important for the continuous movement of water from the root through the plant to the surrounding atmosphere during transpiration.

In contrast to the stable situation for metals, water under tension is actually in a metastable state. If gas bubbles form in the water under tension in the xylem vessels, the column of liquid can be ruptured. Thus the introduction of another phase can disrupt the metastable state describing water under tension. Minute air bubbles sometimes spontaneously form in the xylem as the external temperature rises. These usually adhere to the walls of the xylem vessel (or the pores of the perforation plate), and the gas in them slowly redissolves. However, if they grow large enough or a number of small bubbles coalesce, that xylem vessel could cease functioning. Moreover, freezing of the solution in xylem vessels can lead to bubbles in the ice (the solubility of air is quite low in ice), and these bubbles could then interrupt the water columns upon thawing. Some plants are in fact damaged by freezing and thawing of xylem sap and the consequent loss of water continuity in the xylem vessels (see Zimmermann and Brown).

Electrical properties. Another important physical characteristic of water is its extremely high dielectric constant, again, a consequence of its molecular structure. A high dielectric constant for a solvent lowers the electrical forces between charged solutes. To quantify the magnitude of electrical effects occurring in a fluid, let us consider two ions having charges Q_1 and Q_2 and separated from each other by a distance r . The electrical force exerted by one ion on the other is expressed in Coulomb's law,

$$\text{electrical force} = \frac{Q_1 Q_2}{4\pi\epsilon_0 D r^2} \quad (2.3)$$

where ϵ_0 is a proportionality constant known as the permittivity of a vacuum (electrical units are briefly discussed in App. II). In Equation 2.3, D is a dimensionless quantity called the dielectric constant. It equals unity in a vacuum and is 1.00058 in air at 0° at a pressure of 760 mm Hg.

Any substance composed of highly polar molecules, such as water, gener-

ally has a high dielectric constant. Specifically, D for water is 80.2 at 20° and 78.4 at 25°, which are extremely high values for dielectric constants of liquids. By contrast, the dielectric constant of the nonpolar liquid hexane is only 1.87—such a low value is typical of many organic solvents. Based on these two vastly different dielectric constants and Equation 2.3, we can calculate that the attractive electrical force between ions such as Na^+ and Cl^- is $(80.2/1.87)$, or 43 times greater in hexane than in water. The much stronger attraction between Na^+ and Cl^- in hexane reduces the amount of NaCl that will dissociate, compared with the dissociation of this salt in aqueous solutions. Stated another way, the much weaker electrical forces in water, compared with those in organic solvents, permit a larger concentration of ions to be in solution. Water is thus a good solvent for charged particles.

The electrostatic interaction between ions and water partially cancels or screens out the local electrical fields of the ions. Cations attract the negatively charged oxygens of the water molecule, while anions reciprocally attract its positively charged hydrogen atoms. The oriented water molecules around the charged particles produce local electrical fields opposing the fields of the ions. The resultant screening diminishes the electrical interaction between the ions and allows more of them to remain in solution. This is the molecular basis for the high dielectric constant of water.

The energy for the interaction between water and nonpolar molecules is usually less than the energy required to break water-water hydrogen bonds. Nonpolar compounds are therefore not very soluble in water. Certain substances such as detergents, phospholipids, and proteins can have both a nonpolar and also a polar region in the same molecule. In aqueous solutions these compounds can form aggregates, called *micelles*, in which the nonpolar groups of the molecules are in the center, while the charged or polar groups are external and interact with water. The lack of appreciable attractive electrostatic interaction between polar or charged species and the nonpolar (hydrophobic) regions of membrane lipids underlies the ability of biological membranes to limit the passage of such solutes into and out of cells and organelles.

Chemical potential

The chemical potential of species j is a thermodynamic way of quantitatively describing the free energy associated with it and available for performing work. Since there are many concepts to be mastered before understanding

such a statement, we will first briefly explore the concept of free energy (further details are presented later in this chapter, on pp. 261–263, and in App. VII). We can then introduce specific terms in the chemical potential of species j and, finally, consider the extremely important case of water.

Free energy and chemical potential. Plants and animals require a continual input of free energy. If we were to remove the sources of free energy, organisms would drift toward equilibrium and consequent cessation of life. The ultimate source of free energy is the sun. Photosynthesis converts its plentiful radiant energy into free energy, which is stored first in intermediate energy “currencies” like ATP and then in the altered chemical bonds which result when CO_2 and H_2O react to form a carbohydrate and O_2 . When the overall photosynthetic reactions are reversed during respiration, the available free energy is reconverted into suitable energy currencies like ATP. In turn, such free energy is used to perform biological work such as transporting amino acids into cells, pumping blood, powering the electrical machinery of the brain, or lifting weights. For every such process on both a molecular and a global scale the free energy decreases. In fact, the structure of cells, as well as that of ecosystems, is governed by the initial supply of free energy and by the inexorable laws of thermodynamics which describe the expenditure of that free energy.

Instead of viewing the whole universe, the thermodynamicist focuses his attention on some small part of it referred to as a “system.” Such a system can be potassium ions dissolved in water, a plant cell, a leaf, or even a whole plant. Something happens to the system; the potassium concentration is increased, some protein is made in a cell, a leaf abscises, or water moves up a tree. We then say that the system changes from state A to state B (the change may of course be brought about in many different ways). The minimum amount of work needed to cause the change from A to B can be defined as the free energy increase associated with it. Alternatively, the free energy decrease in going from state B back to state A represents the maximum amount of work which can be derived from the transition. (If there were no frictional or other irreversible losses, we could do without the words “minimum” and “maximum.”) Thus, the limits to the work done on or by a system when it undergoes a transition from one state to another concern changes in free energy. Knowledge of the free energy under one condition compared with another allows us to predict the direction of spontaneous change or movement—a spontaneous change in a system at constant temperature and pressure always proceeds in the direction of decreasing free energy.

Most systems of interest in biology are subject to a constant pressure

(atmospheric) and remain at constant temperature, at least for short periods. In discussing the energetics of processes at constant temperature and pressure, the appropriate quantity is known as the *Gibbs free energy*. The Gibbs free energy has a very useful property in that it always decreases for a spontaneous process at constant temperature and pressure. Under such conditions, the decrease in Gibbs free energy equals the maximum amount of energy available for work, while if it increases for some transition, the change in Gibbs free energy represents the minimum amount of work required. (We will write down an equation for the Gibbs free energy in Ch. 6, and App. VII deals with a number of formal matters associated with its use.) For our present purposes, we will simply note that the Gibbs free energy of an entire system is made up of additive contributions from each of the species present. We will therefore shift our emphasis to the individual components of the system and the manner in which their free energy can be described.

It turns out that we can assign to every chemical component in a system a free energy per mole of that species. The quantity is called the *chemical potential* of species j and is given the symbol μ_j . We can view μ_j as a property of species j that tells how that species will react to a given change, e.g., a transition of the system from state A to state B . During the transition the chemical potential of species j changes from μ_j^A to μ_j^B . If μ_j^B is less than μ_j^A , then the free energy per mole of species j decreases. Such a process can take place spontaneously as far as species j is concerned—water flowing downhill is an example of such a spontaneous process. (We are restricting our attention to changes that occur when the system is at a constant temperature and subjected to a constant pressure.) In principle, we can harness the spontaneous change to do work. The maximum amount of work that can be done per mole of species j is $\mu_j^A - \mu_j^B$ (Fig. 2.3). Suppose now that μ_j^B equals μ_j^A . As far as species j is concerned, no work is involved in the transition from state A to state B —species j is then at equilibrium (cf. Fig. 2.3). (We will show later that not only are living systems as a whole far from equilibrium, but also many chemical species are not in equilibrium across cellular membranes.) Finally, let us consider the case where μ_j^B is greater than μ_j^A . A change which increases the chemical potential of a substance can only occur if some other change occurs in the system—this other change supplying the free energy required. The pumping of blood along arteries using ATP to cause contraction of the heart muscles is an instance where the increase in chemical potential of a mole of water in the blood is accompanied by an even larger decrease in the free energy associated with ATP (we will consider ATP in detail in Ch. 6). The minimum

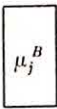
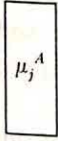
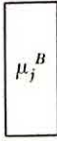
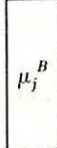
Initial chemical potential	Final chemical potential	Comment
		Transition can occur spontaneously as far as species j is concerned; maximum work available per mole of species j equals $\mu_j^A - \mu_j^B$
		Equilibrium
		Change will not occur unless free energy at least equal to $\mu_j^B - \mu_j^A$ is supplied per mole of species j

Figure 2.3

Possible changes in the chemical potential of species j which can accompany a transition from initial state A to final state B —as might occur in a chemical reaction or in crossing a membrane. The heights of the bars representing μ_j correspond to the relative values of the chemical potential.

amount of work that must be done per mole of species j to cause the energetically uphill transition is $\mu_j^B - \mu_j^A$ (Fig. 2.3).

The condition for a spontaneous change, namely a decrease in chemical potential, has important implications for discussing fluxes from one region to another. In particular, we can use the chemical potential difference between two locations as a measure of the “driving force” for the movement of that component. Thus, the bigger the chemical potential difference, $\mu_j^A - \mu_j^B$, the faster the spontaneous change takes place—in this case, the larger the flux of species j from region A to region B .

The chemical potential of a substance clearly depends on its chemical composition, but it is also influenced by other factors. Here we shall argue in an intuitive way about what environmental factors might influence μ_j .

To begin with, we note that chemical potential depends on the "randomness" (entropy) of the system, and concentration is a quantitative description of this randomness. For example, the passive process of diffusion discussed in Chapter 1 describes neutral molecules spontaneously moving from some region to another where their concentration is lower. In more formal terms, diffusion of species j actually proceeds in the direction of decreasing μ_j , which in this case is the same as that of decreasing concentration. The concept of chemical potential was thus implicit in the development in Chapter 1 of Fick's equations. At the same time, the importance of concentration gradients, or differences, in leading to the movement of a substance was also established in the discussion of diffusion. Here we will present the effect of concentration on chemical potential in a somewhat more sophisticated manner, using that part of concentration which is thermodynamically active.

Chemical potential also depends on pressure, which for situations of interest in plant physiology means hydrostatic pressure. The existence of pressure gradients can cause movements of fluids—the flow of crude oil in long-distance pipelines, blood in arteries, and sap in xylem vessels—which is another way of saying that pressure differences can affect the direction for spontaneous changes.

Electrical potential also affects the chemical potential of charged particles, hence it must be considered when predicting the direction of their movement. It is a matter of definition that work must be done to move a positively charged particle to a higher electrical potential. Accordingly, if an electrical potential difference is imposed across an electrolyte solution containing a uniform concentration of ions throughout, then cations will spontaneously move in one direction (toward the cathode, i.e., to regions of lower electrical potential), while anions will move in the opposite direction (toward the anode). It is clear, then, that in order to describe the chemical potential of a charged species we must include an electrical term in μ_j .

Another contributor to chemical potential is gravity. We can readily appreciate that position in a gravitational field affects μ_j , inasmuch as work must be done to move the substance upward. Although the gravitational term can be neglected for ion and water movements across plant cells and membranes, it is important for water movement in a tall tree.

In sum, the chemical potential of some species should depend on its concentration, the pressure, the electrical potential, and gravity. We can compare the chemical potentials of a species on two sides of a barrier in order to decide whether or not the species is in equilibrium. If μ_j is the same on the two sides, we would not expect a net movement of species j to occur

spontaneously across the barrier. We will use the relative values of the chemical potential of species j at various locations to predict the directions for spontaneous movement of that chemical substance (viz., toward lower μ_j), just as comparison of temperatures can be used to predict the direction for heat flow (toward lower T). Finally, we will find that $\Delta\mu_j$ from one region to another gives a convenient measure of the driving force on species j .

Analysis of chemical potential. For convenience and also because it has proved experimentally valid, we will represent the chemical potential of any species j by the following sum of the various components into which it can be analyzed:

$$\mu_j = \mu_j^* + RT \ln a_j + \bar{V}_j P + z_j F E + m_j g h \quad (2.4)$$

One measure of the elegance of a mathematical expression is the amount of usefulness or information which it contains. Based on such a criterion, Equation 2.4 truly represents one of the most elegant relations in all of biology. After defining and describing the various contributions to μ_j indicated in Equation 2.4, we will consider in greater detail the various terms for the important special case where species j is water.

Chemical potential, like electrical or gravitational potentials, is a relative quantity. By this we mean that it must be expressed relative to some arbitrary level. Thus, an unknown additive constant, or reference level, μ_j^* , is included in Equation 2.4. Since it contains an unknown constant, the actual value of chemical potential is not determinable. For most applications of chemical potential in physiology, we are interested in the difference in the chemical potential between two particular locations, in which case only relative values of the chemical potential are important anyway. The arbitrariness of μ_j^* is of little consequence because it is added to each of the chemical potentials under comparison. Therefore, μ_j^* cancels out when the chemical potential in one location is subtracted from that in another in order to obtain the chemical potential difference between the two locations. Figure 2.3 further illustrates the usefulness of considering differences in chemical potential instead of the actual value of μ_j . (We will return to μ_j^* when we consider μ_j in the standard state.) Finally, we note that the units of μ_j and of μ_j^* are energy per mole of the substance, e.g., joules/mole or cal/mole.

In certain cases we can adequately describe the chemical properties of species j by using the concentration of that solute, c_j . Owing to molecular interactions, however, this usually requires that the total solute concentration be low. Molecules of solute species j interact with each other as well as with other solutes in the solution, and this influences the behavior of species j . Such intermolecular interactions increase as the solution becomes more

concentrated. The use of concentrations for describing the thermodynamic properties of some solute thus indicates an approximation, except in the limiting case of infinite dilution where interactions between solute molecules are negligible. Where exactness is required, *activities*—which may be regarded as “corrected” concentrations—are employed. Consequently, for general thermodynamic considerations, such as Equation 2.4, the influence of the amount of a particular species j on its chemical potential is not handled by its concentration but by the activity of that species, a_j . The activity of solute j is related to its concentration by means of an activity coefficient, γ_j :

$$a_j = \gamma_j c_j \quad (2.5)$$

The activity coefficient is usually less than unity, since the thermodynamically *effective* concentration, or *activity* of a species is generally less than is its actual concentration.*

For an ideal solute, γ_j is one and the activity of species j then equals its concentration. This condition can be approached for real solutes in certain dilute aqueous solutions, especially for neutral species. Activity coefficients for charged species can be appreciably less than unity because of the importance of electrical interactions (see pp. 95–97).

The activity of a solvent is defined in a manner different from that for a solute. The a_j of a solvent is defined as $\gamma_j N_j$, where N_j is the mole fraction of the solvent (see p. 65). An ideal solvent has γ_{solvent} equal to 1, while an ideal solution has all activity coefficients equal to unity.

In the expression for chemical potential, Equation 2.4, the term involving the activity appears as $+ RT \ln a_j$. Therefore, the greater the activity of species j —loosely speaking, the higher its concentration—the larger will be its chemical potential. The appearance of the logarithmic form can be “justified” in a number of different ways, all of which are based on agreement with empirical observations. (The particular justifications invoked in this text are given on pp. 105–106 and 459. It should be noted that the appearance of a logarithmic term in energy expressions is not without precedent. For example, the expression for work done in compressing a gas also leads to a logarithmic form.) The factor RT multiplying $\ln a_j$ in Equation 2.4 gives the activity term the units of energy per mole.

The term $\bar{V}_j P$ in Equation 2.4 represents the effect of pressure on chemical potential. Since essentially all measurements in plant physiology are made

* See Bull or Edsall and Wyman for the very special cases where γ_j can be greater than unity for high concentrations of certain ions.

on systems subjected to atmospheric pressure, it is convenient to define P as the pressure in excess of this, and we will adopt such a convention here. $\bar{V}_j$ is the differential increase in volume of a system when a differential amount of species j is added, other species, temperature, pressure, electrical potential, and gravitational position remaining constant:

$$\bar{V}_j = \left(\frac{\partial V}{\partial n_j} \right)_{n_i, T, P, E, h} \quad (2.6)$$

The subscript n_i on the partial derivative in Equation 2.6 means that the numbers of moles of all species present, other than species j , are held constant when the derivative is taken—the four other subscripts are included to remind us of the additional parameters which must be held constant.

$\bar{V}_j$ is called the *partial molal volume* of species j . (μ_j is actually the partial molal Gibbs free energy, $(\partial G/\partial n_j)_{n_i, T, P, E, h}$, as we will discuss in Ch. 6 and App. VII.) The partial molal volume of a substance is often nearly equal to the volume of a mole of that species, but because there is in general a slight change in total volume when substances are mixed, $\bar{V}_j$ is not exactly equal to it.* We will indicate that the value of $\bar{V}_j$ for water is about 18 cm³/mole when we specifically consider the chemical potential of water.

Since work is often expressed as pressure times change in volume, note that $\bar{V}_j P$ has the correct units for μ_j of energy per mole ($\bar{V}_j$ is the volume per mole). To help justify the form of the pressure term, let us imagine that the solution containing species j is built up by adding small volumes of species j while the system is maintained at a constant pressure P . The work done to add a mole of species j would then be the existing pressure times some volume change of the system which characterizes a mole of that species, viz., $\bar{V}_j$. (In App. VII the inclusion of the $\bar{V}_j P$ term in the chemical potential of species j is "justified" in a more formal manner.)

The influence of electrical potential on the μ_j of an ion is handled by the term $z_j F E$ in Equation 2.4, where z_j is an integer representing the charge number of species j , F is a constant known as the Faraday (see p. 93), and E is electrical potential. Because water is uncharged ($z_w = 0$), the electrical term does not contribute to its chemical potential. However, electrical potential is of central importance when discussing ions and also the origin of membrane potentials, both of which are examined in detail in Chapter 3, where we will explicitly consider the $z_j F E$ term. The full

* The addition of small concentrations of certain salts can actually cause a solution to contract—a phenomenon known as electrostriction—which in this special case would result in a negative value for $\bar{V}_j$.

expression for chemical potential, μ_j , given by Equation 2.4 including z_jFE , is often referred to as *electrochemical potential* when discussing the properties of charged particles.

For completeness, the gravitational term m_jgh is included in Equation 2.4. It is apparent that to raise an object of mass m_j per mole to a vertical height h requires an amount of work m_jgh , where g is the gravitational acceleration (close to 980 cm/sec², but see App. II for details). In the case of water, which is the primary concern of this chapter, m_w is 18.016 g/mole. The gravitational term in the chemical potential is not only important for the fall of rain, snow or hail, but also it affects the percolation of water downward through porous soil and the upward movement of water in a tree. The gravitational term m_jgh can have units of (g/mole)(cm/sec²)(cm) or (g-cm²/sec²)/(mole), which is ergs/mole. (Conversion factors between ergs, joules, cal, and other energy units are given in App. III.)

Standard state. The additive constant term μ_j^* in Equation 2.4 is actually the chemical potential of species j for a suitable reference state. From the preceding definitions of the various quantities involved, this reference state is attained when the activity of species j is unity ($RT \ln a_j = 0$), the hydrostatic pressure equals atmospheric pressure ($\bar{V}_jP = 0$), the species is uncharged or the electrical potential is zero ($z_jFE = 0$), we are at the zero level for the gravitational term ($m_jgh = 0$), and the temperature equals that for the system under consideration. Under these conditions, μ_j equals μ_j^* (Eq. 2.4).

As suggested on p. 60, an activity of unity is defined in different ways for the solute and the solvent. To describe liquid properties, such as the dielectric constant, the heat of vaporization, and the boiling point, the most convenient standard state is that of the pure solvent. For a solvent, a_{solvent} equals γ_{solvent} times N_{solvent} , hence the activity is unity when the mole fraction N_{solvent} is unity ($\gamma_{\text{solvent}} = 1$ for pure solvent). For example, the standard reference state for water is pure water at atmospheric pressure and at the temperature and gravitational level of the system under consideration. Water has an activity of unity when N_w as given by Equation 2.8 is one—the concentration of water is then (1000 g of solvent)/(18.016 g/mole), or 55.5 molal. The accepted convention for a solute, on the other hand, is that a_j is unity when $\gamma_j c_j$ equals 1 molal. For example, if γ_j equals one, a solution that has a 1 molal concentration of solute j has an activity of 1 molal for that solute. Thus, the standard state for an ideal solute is when its concentration is 1 molal, in which case $RT \ln a_j$ is zero.

The value of the chemical potential for a solute in the standard state, μ_j^* , actually depends on the solvent. In order to be explicit, let us consider

a two-phase system consisting of water with an overlying layer of olive oil. It turns out that μ_j^* for a polar molecule is smaller for an aqueous solution (where the species readily dissolves) than it is for an organic phase such as olive oil (where it is not as soluble). If we put the polar solute into our system, shake the water and olive oil together, and wait for the two phases to separate out again, the solute concentration will be higher in the bottom or water phase. Let us next analyze this event using symbols, where phase *A* represents water and phase *B* is olive oil. Waiting for the phase separation is equivalent to waiting for the attainment of equilibrium, and so μ_j^A is then equal to μ_j^B . In the present case this means that $\mu_j^{*,A} + RT \ln a_j^A$ equals $\mu_j^{*,B} + RT \ln a_j^B$ by Equation 2.4. But the polar solute is more soluble in water than it is in olive oil, hence $RT \ln a_j^A$ is greater than $RT \ln a_j^B$. Because we are at equilibrium, this means that $\mu_j^{*,A}$ must be less than $\mu_j^{*,B}$, as we already indicated. Such a two-phase separation procedure is actually followed in determining the olive oil-water partition coefficient, a_j^B/a_j^A (which is essentially the same as c_j^B/c_j^A for dilute solutions). Moreover, since the diffusion of many substances across membranes is correlated with their lipid solubility, as we mentioned in Chapter 1, such partition coefficients (p. 28) are of great utility in discussing the movement of solutes into and out of cells or organelles.

Hydrostatic pressure. Because of the rigid cell walls, fairly large hydrostatic pressures can exist in plant cells, while such pressures in animal cells generally are relatively small. In addition to being involved in the support of the plant, hydrostatic pressures are important for the movement of water and solutes in the xylem and probably also in the phloem. The term expressing the effect of pressure on the chemical potential of water is $\bar{V}_w P$ (cf. Eq. 2.4), where $\bar{V}_w$ is the partial molal volume of water and *P* is the hydrostatic pressure in the aqueous solution in excess of the ambient atmospheric pressure. The approximate value of $\bar{V}_w$ can be estimated using the following argument. When 1 mole (18.0 g) of water is added to water, the volume increases by 18.0 cm³, since the density of water at 20° is 0.998 g/cm³. In other words, $\bar{V}_w$ is 18.0 cm³/mole in the case of pure water. Although $\bar{V}_w$ (defined according to the partial derivative given in Eq. 2.6) can be influenced by the various solutes actually present, it generally is close to 18.0 cm³/mole for a dilute solution, a value that we will use for calculations in this book.

Various units are used for expressing pressures, atmospheres and bars being commonly employed in plant physiology. A pressure of one atmosphere is defined as that pressure which can support either a column of mercury 76.0 cm high or a column of water 10.3 m high. However, most

current research concerning the water relations of plants uses pressures in bars. A bar is 10^6 dynes/cm², which equals 0.987 atmosphere. (An extensive list of conversion factors for bars, atmospheres, and other pressure units is given in App. III, which also includes values for related quantities such as RT expressed in units employing both atmospheres and bars.) Pressure is force per unit area, which is dimensionally the same as energy per unit volume, e.g., one bar is 0.100 joule/cm³ or 0.0239 cal/cm³. Since $\bar{V}_w$ has the units of cm³/mole, the quantity $\bar{V}_w P$ and hence μ_w itself can be expressed in joules/mole or cal/mole.

Water activity and osmotic pressure. The presence of solutes in an aqueous solution tends to decrease the activity of water (a_w). As a first approximation, we can view the decrease in a_w as more and more solutes are added as simply a dilution effect. In other words, the concentration (or mole fraction) of water becomes less when the water molecules are displaced by those of the solute. As its activity thus decreases, we would expect a lowering of the chemical potential of water, as does indeed occur (cf. the $RT \ln a_j$ term in μ_j). We also know that the presence of solutes can lead to an osmotic pressure (π) in the solution. An increase in the concentration of solutes raises the osmotic pressure, indicating that π and a_w change in opposite directions. In fact, the osmotic pressure and water activity are related, a fundamental definition of π being

$$RT \ln a_w = -\bar{V}_w \pi \quad (2.7)$$

where the subscript w refers to water. As solutes are added, a_w decreases from its value of unity for pure water, $\ln a_w$ is therefore negative, and π (defined by Eq. 2.7) positive, which is consistent with the arguments presented above. Equation 2.7 also indicates that $RT \ln a_w$, a term appearing in the chemical potential of water (cf. Eq. 2.4), can be replaced by $-\bar{V}_w \pi$.

Unfortunately there is much variation on the use of the terms "osmotic pressure" and "osmotic potential," as well as the algebraic sign of the quantity. Osmotic pressures were originally measured using an osmometer, a device employing a membrane that in the ideal situation was permeable to water but not to any of the solutes present. When pure water was placed on one side of the membrane and some solution on the other, there was a net diffusion of water toward the side with the solutes. To counteract this tendency and establish equilibrium, a hydrostatic pressure had to be applied to the solution side. This pressure was called the *osmotic pressure*—it depended of course on the presence of the solutes. Does an isolated solution have an osmotic pressure? In the sense of requiring an applied hydrostatic pressure to maintain equilibrium, the answer is no. Yet the same solution

when placed in an osmometer can manifest an osmotic pressure. Thus, some physical chemistry texts have found it convenient to say that a solution has an osmotic *potential*—i.e., it could show an osmotic pressure if placed in an osmometer—and that osmotic potentials are a property characteristic of solutions (cf. Morris). Many plant physiology books have come to a similar conclusion, e.g., Salisbury and Ross, but have defined the *negative* of the same quantity as the osmotic potential, in order to have the water potential (a quantity we will introduce shortly) depend directly on the osmotic potential and not on its negative. We will not try to resolve this dilemma. Instead, we will consistently refer to π defined by Equation 2.7 as osmotic pressure (cf. Van Holde). In principle, it is always possible to measure π as a pressure by using an osmometer.

Using another “colligative” property is a direct and convenient way of evaluating osmotic pressure and water activity as defined by Equation 2.7. The four colligative properties of a solution—those that depend on the concentration of solutes regardless of their nature, at least in a dilute enough solution—are the freezing point depression, the boiling point elevation, the lowering of the vapor pressure of the solvent, and osmotic pressure. Thus, if the freezing point of cell sap is measured, $\pi^{\text{cell sap}}$ can be calculated using the freezing point depression of 1.86° for a 1 molal solution and the *Van't Hoff relation*.

The Van't Hoff relation. For many purposes in plant physiology it is convenient to relate osmotic pressure directly to the concentration of solutes instead of expressing π in terms of the water activity, a_w , as is done in Equation 2.7. In general, the greater the concentration of solutes, the more negative $\ln a_w$ becomes and the larger is the osmotic pressure. Thus, some way of expressing a_w in terms of the properties of the solutes would seem to be an appropriate approach for expressing π as a function of the various solute concentrations. The ensuing derivation not only will show how a_w can be so expressed, but will also serve to indicate the many approximations necessary in order to achieve a rather simple expression for π .

The activity of pure water is unity, while in general a_w equals $\gamma_w N_w$, where γ_w is the activity coefficient of water and N_w is its mole fraction. The mole fraction of a species is simply the ratio of the number of moles of that species to the total number of moles of all species in the system. Thus, N_w is given as:

$$N_w = \frac{n_w}{n_w + \sum_j n_j} = 1 - \frac{\sum_j n_j}{n_w + \sum_j n_j} \quad (2.8)$$

keeping device for handling interfacial interactions. To help make this statement more meaningful, we will briefly consider the influence on the chemical potential of water of liquid-solid interfaces at the surfaces of colloids.*

When water molecules are associated with interfaces such as those provided by the surfaces of colloidal particles suspended in an aqueous solution, they have less of a tendency either to react chemically in the bulk solution or to escape into a surrounding vapor phase. The presence of interfaces thus lowers the thermodynamic activity of the water (a_w), especially near the surfaces of the colloids. But the presence of solutes also lowers water activity, an effect that can be expressed by Equation 2.10. As a useful first approximation, we can consider that these two different effects lowering water activity are additive in a solution which contains both ordinary solutes and colloids. Osmotic pressure (π) depends on the actual activity of water regardless of the reason for a_w being different from unity, i.e., π still equals $-(RT/\bar{V}_w) \ln a_w$, as it must by Equation 2.7. Combining these various ideas (and recalling that $a_w = \gamma_w N_w$) we can write the following relation:

$$\pi = -\frac{RT}{\bar{V}_w} \ln \gamma_w N_w = -\frac{RT}{\bar{V}_w} \ln \gamma_w - \frac{RT}{\bar{V}_w} \ln N_w = \tau + \pi_s \quad (2.11)$$

where τ is a matric pressure resulting from the water-solid interactions at the surfaces of the colloids.

Note that π_s in Equation 2.11 is to be identified with the osmotic pressure of all solutes, including the *concentration* of the colloids, as represented by Equation 2.10 ($\pi_s \cong RT \sum_j c_j$). In other words, we were actually dealing only with $-(RT/\bar{V}_w) \ln N_w$ in the derivation of π_s , since γ_w was then set equal to unity. For an exact treatment when many interfaces are present—e.g., in the cytoplasm of a typical cell—we cannot set γ_w equal to unity, since the activity of water, and hence the osmotic pressure (π), is strongly affected by the proteins and other colloids present. In such a case, Equation 2.11 suggests a simple way in which matric pressure may be related to the reduction of the activity coefficient of water caused by the interactions at interfaces. (Note that if we let $\tau = -(RT/\bar{V}_w) \ln \gamma_w$, then γ_w less than unity leads to a positive τ .) Equation 2.11 should not be viewed as a relation defining τ , rather, it is one example where it might be useful to represent

* "Colloid" is a generic term for the dispersion of solid particles approximately 0.002 to 1 μm in diameter suspended in a liquid, i.e., large, but generally submicroscopic particles including proteins, ribosomes, and even some organelles.

interfacial interactions by a separate term which can be added to π_s , the effect of the solutes on π .

Although π and a_w may be the same throughout some system, both π_s and τ in Equation 2.11 may vary. For example, water activity in the bulk of the solution may be predominantly lowered by the presence of the solutes, while at or near the surface of colloids the main factor decreasing a_w from one could be the interfacial attraction and binding of water. This latter effect does not change the mole fraction of water, but does reduce its activity coefficient, γ_w .

Other areas of plant physiology where matric potentials have been invoked are in descriptions of the chemical potential of water in soil or in cell walls. Soil matric potentials will be mentioned in Chapter 8, while cell walls will be further considered at the end of this chapter. The interfacial interactions between water and the many surfaces lining the interstices of the cell wall lead to a tension in the cell wall water. Such a tension is simply a negative hydrostatic pressure, i.e., P is less than zero. Although it is not necessary, P is sometimes used to refer only to positive pressures, while negative P in cell wall water is often defined as a positive matric potential. Some recent books define this same negative hydrostatic pressure in water in cell wall interstices or between soil particles as a negative matric potential. It is far more straightforward to refer to the quantity which reduces μ_w in such pores as a negative P .

Water potential. From the definition of chemical potential (Eq. 2.4) and the formal expression for osmotic pressure (Eq. 2.7), we can write the chemical potential of water (μ_w) as

$$\mu_w = \mu_w^* - \bar{V}_w \pi + \bar{V}_w P + m_w g h \quad (2.12)$$

where the electrical term ($z_j F E$) is not included, since water carries no charge ($z_w = 0$). The quantity $\mu_w - \mu_w^*$ has proved to be of considerable importance for discussing the water relations of plants. It represents the work involved in moving one mole of water from some point in a system (at constant pressure and temperature) to a pool of pure water at atmospheric pressure, at the same temperature as the system under consideration, and at the zero level for the gravitational term. More to the point, a difference between two locations in the value of $\mu_w - \mu_w^*$ indicates that water is not in equilibrium, hence there will be a tendency for water to flow toward the region where $\mu_w - \mu_w^*$ is lower.

A quantity proportional to $\mu_w - \mu_w^*$ and increasingly used in studies of plant water relations is the so-called *water potential*, Ψ . This is conveniently

defined as

$$\Psi = \frac{\mu_w - \mu_w^*}{\bar{V}_w} = P - \pi + \rho_w gh \quad (2.13)$$

which follows directly from Equation 2.12 plus the identification of $m_w/\bar{V}_w$ with the density of water, ρ_w . Equation 2.13 indicates that an increase in hydrostatic pressure raises the water potential, while an increase in osmotic pressure lowers it. We will consider the values of Ψ on both sides of cellular and subcellular membranes in the next two sections.

Some older terminology describing plant water relations can be identified from Equation 2.13. For example, $-\Psi$ is the quantity called “diffusion pressure deficit” or “suction pressure” (actually, the term $\rho_w gh$ was nearly always neglected). Although these expressions are quite colorful, the terms *water potential* (Ψ) and *chemical potential of water* (μ_w) are increasingly being used in biology and other disciplines, and we will use them in this text. Hydrostatic pressure (P) is often referred to as “turgor pressure” in considerations of the protoplast, or, from a complementary point of view, as “wall pressure” in considerations of the pushing of the cell wall back against the protoplast.

We have already mentioned that work must be performed to raise an object in the gravitational field of the earth. Thus, vertical position also affects the chemical potential, consequently, the term $\rho_w gh$ must be included in the water potential given by Equation 2.13, at least when water moves an appreciable distance vertically in the gravitational field. In plant physiology the magnitude of $\rho_w g$ is conveniently expressed as 0.098 bar/m—if water moves 10 m vertically upward in a tree, the gravitational contribution to the water potential is increased by about 1 bar. For most applications in this text, such as consideration of chemical reactions or the crossing of membranes, there is no change in vertical position and the gravitational term will generally be omitted from μ_j and Ψ .

Central vacuole and chloroplasts

The water relations of both the large central vacuole and the chloroplasts will be considered next, using the water potential as defined above. We will focus our attention on the situation where there is no net flow of water across the limiting membranes surrounding these subcellular compartments and for the special case of nonpenetrating solutes. (The more general case of penetrating solutes will be discussed in Ch. 3 after a consideration of the

properties of solutes and the introduction of concepts from irreversible thermodynamics.)

The central vacuole occupies up to 90% of the volume of a mature plant cell, which means that under such circumstances most of the cellular water is in the vacuole. The vacuolar volume is generally from 10^4 to $10^5 \mu\text{m}^3$ for the mesophyll cells in a leaf, but can be much larger in certain algal cells. Because its size is similar to that of the cell, the vacuole cannot be easily removed from the plant cell without rupturing its surrounding membrane, the tonoplast. In other words, procedures which remove the cell wall and open up the plasmalemma usually also break the tonoplast, especially for small cells. Chloroplasts are much smaller than the central vacuole, often having volumes near $30 \mu\text{m}^3$ *in vivo* (although the sizes vary considerably with the plant species). When chloroplasts are carefully isolated, suitable precautions will ensure that their limiting membranes remain intact. Such intact chloroplasts can be placed in solutions having various osmotic pressures and the resulting movement of water into or out of the isolated plastids can be precisely measured.

Water relations of the central vacuole. In order to predict whether and in what direction water will move, we need to know the value of the water potential in the various compartments under consideration. At equilibrium the water potential is the same in all communicating phases, such as those separated by membranes. For example, when water is in equilibrium across the tonoplast, the value of the water potential is the same in the vacuole as it is in the cytoplasm. No force then drives water across this membrane, and no net flow of water occurs into or out of the vacuole.

The tonoplast most likely does not have an appreciable difference in hydrostatic pressure across it. A higher internal hydrostatic pressure would cause a typical slack (folded) tonoplast to be mechanically pushed outward. The observed lack of motion thus indicates that ΔP is zero across a slack tonoplast. If the tonoplast were taut, ΔP would cause a stress in the membrane, analogous to the cell wall stresses discussed in the last chapter. For a tonoplast 10 nm thick, surrounding a spherical vacuole 10 μm in radius, the stress would be $r\Delta P/2t$, which is $(10 \times 10^{-6} \text{ m})\Delta P/[2(10 \times 10^{-9} \text{ m})]$, or $500 \Delta P$. If the hydrostatic pressure were only 0.01 bar higher in the vacuole compared with the cytoplasm, in a taut tonoplast there would then be a stress of $(500)(0.01)$, or 5 bars. This exceeds the tensile strength of biological membranes—most membranes rupture before a stress of even 1 bar develops in them. Thus, P may well have essentially the same value in the cytoplasm and the vacuole. With this simplifying assumption, and for the equilibrium situation where Ψ is the same in the two phases, Equation 2.13 ($\Psi =$

$P - \pi + \rho_w g h$ where $\Delta h = 0$ across a membrane) gives

$$\pi^{\text{cytoplasm}} = \pi^{\text{vacuole}} \quad (2.14)$$

where $\pi^{\text{cytoplasm}}$ is the osmotic pressure in the cytoplasm and π^{vacuole} that in the central vacuole.

The vacuole appears to be a relatively homogeneous aqueous phase, while the cytoplasm is a more complex phase containing many colloids and membrane-bounded organelles. Since the vacuole contains few colloidal or other interfaces, any matric potential in it is probably negligible compared with the osmotic pressure resulting from the vacuolar solutes. Expressing osmotic pressure by Equation 2.11 ($\pi = \pi_s + \tau$) and assuming that τ^{vacuole} is negligible, we can replace π^{vacuole} in Equation 2.14 by π_s^{vacuole} , i.e., the decrease in vacuolar water activity is due almost solely to the presence of solutes. On the other hand, water activity in the cytoplasm is presumably considerably lowered both by solutes and by the presence of interfaces. In other words, individual water molecules in the cytoplasm are never very far away from proteins, organelles, or other colloids, so that interfacial interactions can appreciably affect $a_w^{\text{cytoplasm}}$. In terms of Equation 2.11, this means that both $\pi_s^{\text{cytoplasm}}$ and $\tau^{\text{cytoplasm}}$ contribute to $\pi^{\text{cytoplasm}}$. Hence, Equation 2.14 indicates that $\pi_s^{\text{cytoplasm}} + \tau^{\text{cytoplasm}}$ equals π_s^{vacuole} . Since $\tau^{\text{cytoplasm}}$ is a positive quantity, we conclude that at equilibrium, the osmotic pressure in the vacuole due to solutes, π_s^{vacuole} , must be larger than $\pi_s^{\text{cytoplasm}}$. Equation 2.14 leads then to the prediction that the vacuole has a higher concentration of osmotically active solutes than does the cytoplasm.

There are various speculations concerning the role of the large central vacuole in plant cells. The vacuole might act as a storage site for metabolites, ions, or toxic products. Because of the large central vacuole, the cytoplasm occupies a thin layer around the periphery of the cell (Fig. 1.1). Therefore, for its volume, the cytoplasm has a relatively large surface area (across which diffusion can occur). Vacuoles also provide large, relatively simple compartments in which hydrostatic pressures lead to the cellular turgidity necessary for support of the plant. The actual function of the central vacuole most likely entails a combination of these various possibilities.

Boyle-Van't Hoff relation. The volume of a chloroplast or other membrane-bounded body changes in response to variations in the osmotic pressure of the external solution, π° . This is a consequence of the properties of membranes, which generally allow water to move readily across them, at the same time restricting the passage of certain solutes, such as sucrose. For example, if π° outside a membrane-bounded aqueous compartment were raised, we would expect that water would flow out across the membrane

to the region of lower chemical potential and thereby decrease the compartment volume, while in general there would be very little movement of solutes in such a case. This differential permeability leads to the "osmometric behavior" characteristic of many cells and organelles. The conventional expression quantifying this volume response to changes in the external osmotic pressure is the *Boyle-Van't Hoff relation*:

$$\pi^o(V - b) = RT \sum_j \varphi_j n_j = RT n \quad (2.15)$$

where π^o is the osmotic pressure of the external solution, V is the volume of the cell or organelle, b is the so-called nonosmotic volume (osmotically inactive volume) and is frequently considered to be the volume of a solid phase within volume V which is not penetrated by water, n_j is the number of moles of species j within $V - b$, the osmotic coefficient (φ_j) is generally construed as a correction factor, and $n = \sum_j \varphi_j n_j$ is the apparent number

of osmotically active moles in $V - b$ (see Dick, Giese, and Nobel).

In this chapter we will derive the Boyle-Van't Hoff relation using the concept of chemical potential, and in Chapter 3 we will extend the treatment by employing irreversible thermodynamics. Although the derived Boyle-Van't Hoff expression will be used only to interpret the osmotic responses of chloroplasts, the equations that will be developed are quite general and can be applied equally well to data obtained with mitochondria, whole cells, or other membrane-surrounded bodies.

The Boyle-Van't Hoff relation applies to the equilibrium situation when equality of the water potential has been achieved across the membranes surrounding chloroplasts exposed to a particular external osmotic pressure. When Ψ^i equals Ψ^o , net water movement across the membrane ceases, and the volume of a chloroplast has a steady value. (The superscript i refers to the inside of the cell or organelle, the superscript o designates the outside.) If we were to measure the chloroplast volume under such conditions, the external solution would generally be at atmospheric pressure ($P^o = 0$ bars). From Equation 2.13 ($\Psi = P - \pi$), the water potential in the external solution is then

$$\Psi^o = -\pi^o \quad (2.16)$$

As is the case for Ψ^o (Eq. 2.16), the water potential inside the chloroplasts depends on osmotic pressure. In addition, the internal hydrostatic pressure (P^i) may be different from atmospheric pressure and should be included

in the expression for Ψ^i . Macromolecules and solid-liquid interfaces inside the chloroplasts can lead to a matric potential, τ^i . To allow for this possibility, the internal osmotic pressure (π^i) will be considered as the sum of the solute and the interfacial contributions, in the manner expressed by Equation 2.11. Using Equation 2.13, Ψ^i is therefore

$$\Psi^i = P^i - \pi^i = P^i - \pi_s^i - \tau^i \quad (2.17)$$

We will now re-express π_s^i and then equate Ψ^o to Ψ^i . For a dilute ideal solution inside the chloroplasts, π_s^i equals $RT \sum_j n_j^i / (\bar{V}_w n_w^i)$ by Equation

2.10. As indicated on p. 67, solutions of real solutes necessitate the introduction of an activity coefficient for solute j , γ_j , which approaches one as the solution becomes more dilute. To handle deviations from ideal behavior for the solutes, n_j^i can thus be replaced by $\gamma_j^i n_j^i$. Making these changes and equating the water potential outside the chloroplast (Eq. 2.16) to that inside (Eq. 2.17), the condition for water equilibrium across the limiting membranes of the chloroplast becomes

$$\pi^o = RT \frac{\sum_j \gamma_j^i n_j^i}{\bar{V}_w n_w^i} + \tau^i - P^i \quad (2.18)$$

Although this expression may look a little frightening, it states only that at equilibrium the water potential is the same on the two sides of the membranes and that we can explicitly recognize various contributors to the two Ψ s involved.

To appreciate the refinements this thermodynamic treatment introduces into the customary expression describing the osmotic responses of cells and organelles, Equation 2.18 should be compared with the conventional Boyle-Van't Hoff relation (Eq. 2.15). The volume of water inside the chloroplast is $\bar{V}_w n_w^i$, since n_w^i is the number of moles of internal water and $\bar{V}_w$ is the volume of one mole of water. This factor in Equation 2.18 can be identified with $V - b$ in Equation 2.15. Instead of being designated the "nonosmotic volume," b is more appropriately called the "nonwater volume," for it includes the volume of the internal solutes including that of colloids. In other words, the total volume (V) minus the nonwater volume (b) equals the volume of internal water ($\bar{V}_w n_w^i$). The osmotic coefficient, φ_j in Equation 2.15, is generally less than 1, as is the activity coefficient of an internal solute, γ_j^i , in Equation 2.18. But γ_j^i cannot simply be equated to φ_j since other factors are also involved (to be considered in Ch. 3). Finally, the possible hydrostatic and matric contributions included in Equation 2.18

are neglected in the usual Boyle-Van't Hoff relation. In short, although certain approximations and assumptions have crept into Equation 2.18 (e.g., that solutes do not cross the limiting membranes, and using dilute solution considerations), it is still a far more satisfactory expression for describing osmotic responses than is the conventional Boyle-Van't Hoff relation.

Osmotic responses of chloroplasts. To illustrate the use of Equation 2.18 in interpreting osmotic data, we will consider osmotic responses of pea chloroplasts suspended in external solutions of various osmotic pressures. Since it is customary to plot the volume V versus the reciprocal of the external osmotic pressure, $1/\pi^o$, certain algebraic manipulations are needed in order to put Equation 2.18 into a more convenient form for discussing such osmometric studies. By transferring $\tau^i - P^i$ to the left side of Equation 2.18 and then multiplying both sides by $\bar{V}_w n_w^i / (\pi^o - \tau^i + P^i)$, $\bar{V}_w n_w^i$ can be shown to equal $RT \sum_j \gamma_j^i n_j^i / (\pi^o - \tau^i + P^i)$. The measured chloroplast volume (V) can be represented by $\bar{V}_w n_w^i + b$, i.e., as the sum of the aqueous and nonaqueous contributions. Hence, V should be equal to $RT \sum_j \gamma_j^i n_j^i / (\pi^o - \tau^i + P^i) + b$, if the modified form of the Boyle-Van't

Hoff relation is obeyed. Figure 2.4 indicates that the volume of pea chloroplasts varies linearly with $1/\pi^o$ over a considerable range of external osmotic pressures. Consequently, $\tau^i - P^i$ occurring in both Equation 2.18 and the above expression must either be negligibly small for pea chloroplasts,

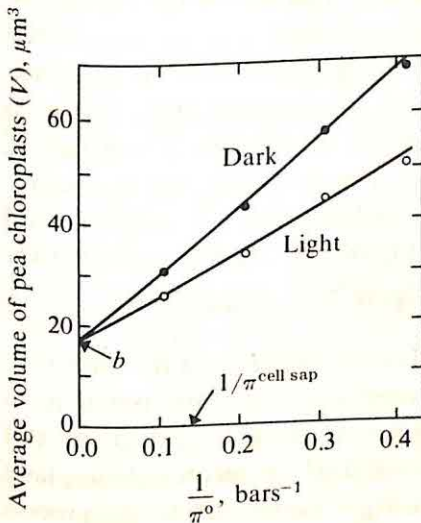


Figure 2.4

Volumes of chloroplasts at various external osmotic pressures, π^o . Pea chloroplasts were isolated from plants in the light or the dark as indicated. (Source: P. S. Nobel, *Biochimica et Biophysica Acta* **172**: 134-143 (1969). Data reprinted by permission.)

compared with the various values of π^0 employed, or perhaps proportional to π^0 . Actually, the situation is rather complicated, since both τ^i and γ_j^i depend on the chloroplast volume. For simplicity, we will ignore these effects and consider only the observed proportionality between V and $1/\pi^0$. In other words, we will assume that $V - b$ (or, $\bar{V}_w n_w^i$) equals $RT \sum_j \gamma_j^i n_j^i / \pi^0$ for

pea chloroplasts. We will return to such considerations in Chapter 3, where we will further refine the Boyle-Van't Hoff relation to include the more realistic case in which solutes can cross the limiting membranes.

The relatively simple measurement of the volumes of pea chloroplasts for various external osmotic pressures can yield a considerable amount of information about the organelles. If we measure the volume of the isolated chloroplasts at the same osmotic pressure they encounter in the cytoplasm, we could then determine the volume the organelles have when in the plant cell. Cell sap expressed from pea leaves was found to have an osmotic pressure of 7.0 bars. Such "sap" comes mainly from the central vacuole, but since we expect $\pi^{\text{cytoplasm}}$ to be essentially equal to π^{vacuole} (Eq. 2.14), $\pi^{\text{cell sap}}$ is about the same as $\pi^{\text{cytoplasm}}$ (a small uncertainty exists inasmuch as during extraction the cell sap can come into contact with water in the cell walls). At an external osmotic pressure of 7.0 bars (indicated by an arrow in Fig. 2.4), pea chloroplasts have a volume of $29 \mu\text{m}^3$ when isolated from illuminated plants, and $35 \mu\text{m}^3$ when isolated from plants in the dark. Since these volumes occur at approximately the same osmotic pressure found in the cell, they are presumably reliable estimates of pea chloroplast volumes *in vivo*.

The data in Figure 2.4 clearly indicate that light affects the chloroplast volume *in vivo*. Although the basis for the size changes is not fully understood, chloroplasts in many plants do have a larger volume in the dark than in the light. The decrease in volume upon illumination of the plants is observed by both phase contrast and electron microscopy as a flattening or decrease in thickness of the chloroplasts. This flattening, which amounts to about 20% of the thickness for pea chloroplasts *in vivo*, is in the vertical direction for the chloroplast depicted in Figure 1.9. We also note that the slopes of the osmotic response curves in Figure 2.4 are equal to $RT \sum_j \gamma_j^i n_j^i$.

The slope is 50% greater for pea chloroplasts from plants in the dark than from those in the light, suggesting that chloroplasts in the dark contain more osmotically active particles. In fact, illumination causes a marked efflux of K^+ and Cl^- from the chloroplasts. Also, some Mg^{++} enters the chloroplasts in the light, perhaps by an exchange of one Mg^{++} for two K^+ . Such a process

would also reduce $\sum_j \gamma_j^i n_j^i$, since only one Mg^{++} would enter for every two K^+ that leave. The entry of Mg^{++} apparently causes its concentration to rise by about 10 mM in the chloroplasts, which may act as a control mechanism for photophosphorylation and CO_2 fixation, both of which depend on magnesium (see Lin and Nobel). Although such speculation takes us far away from our present consideration of water, it indicates the kind of insights that can be obtained from osmometric studies.

The intercept on the ordinate in Figure 2.4 is the chloroplast volume theoretically attained in an external solution of infinite osmotic pressure— $1/\pi^\circ$ equaling zero is the same as a π° of infinity. For such an infinite π° , all of the internal water would be removed ($n_w^i = 0$ moles), and the volume, which is actually obtained by extrapolation, would be that of the nonaqueous components of the chloroplasts.* Thus, the intercept on the ordinate of a V versus $1/\pi^\circ$ plot corresponds to b in the conventional Boyle-Van't Hoff relation (Eq. 2.15). This intercept (indicated by an arrow in Fig. 2.4) equals $17 \mu\text{m}^3$ for chloroplasts both in the light and dark. (The extra solutes in a chloroplast in the dark correspond to less than $0.1 \mu\text{m}^3$ of solids.) Using the chloroplast volumes obtained for a π° of 7.0 bars and $17 \mu\text{m}^3$ for the nonwater volume b , we find that the water content of pea chloroplasts in the dark is $[(35 \mu\text{m}^3 - 17 \mu\text{m}^3)/(35 \mu\text{m}^3)](100)$ or 51% by volume, and in the light $[(29 - 17)/29](100)$ or 41%. These relatively low water contents in the organelles are consistent with the extensive internal lamellar system and the abundance of CO_2 fixation enzymes in chloroplasts, both of which were mentioned in Chapter 1. Thus, osmometric responses of certain cells and organelles can be used to provide information on their fractional water content.

Water potential and plant cells

In this section we will shift our emphasis from a consideration of the water relations of subcellular bodies to those of whole cells, and extend the development to include the case of water fluxes. Whether water enters or leaves a plant cell, and how much, depends on the water potential outside compared with that inside. The external water potential (Ψ°) can often be

* Some uncertainty exists for water that is tightly bound to proteins and other substances. Some of this water presumably remains bound even at the hypothetical infinite osmotic pressures considered here.

varied experimentally, and the direction as well as the magnitude of the resulting water movement will give information about Ψ^i . Moreover, the equilibrium value of Ψ^o can be used to estimate the internal osmotic pressure, π^i .

A loss of water from plants—indeed, sometimes also an uptake—occurs at cell-air interfaces. As we would expect, the chemical potential of water in cells compared with that in the adjacent air determines the direction for net water movement at such locations. Thus, we must obtain an expression for the water potential in a vapor phase, and then relate this Ψ_{wv} to Ψ for the liquid phases in a cell. We will specifically consider the factors influencing the water potential in the component at the plant cell-air interface, viz., the cell wall. We will find that $\Psi^{\text{cell wall}}$ is dominated by a negative hydrostatic pressure resulting from surface tension effects in the cell wall pores.

Incipient plasmolysis. For usual physiological conditions, a positive hydrostatic pressure exists inside a plant cell, i.e., it is under turgor. By suitably adjusting the solutes in an external solution that bathes such a turgid cell, it is possible to reduce P^i to zero and thereby to obtain an estimate of π^i , as the following argument will indicate.

Let us place the cell in pure water ($\pi^o = 0$ bars) at atmospheric pressure ($P^o = 0$ bars). Ψ^o is then zero (ignoring the gravitational term, $\Psi = P - \pi$, Eq. 2.13) while Ψ^i is $P^i - \pi^i$ (the inner phase can be the cytoplasm). If the cell can be assumed to be in equilibrium with this external solution ($\Psi^o = \Psi^i$), P^i must then equal π^i . Suppose that π^o is now gradually raised from its initial zero value. The osmotic pressure outside the cell can be increased for example by adding solute to the external solution. If the cell remains in equilibrium, then π^o equals $\pi^i - P^i$ ($P^o = 0$ bars, since the external solution is at atmospheric pressure). As π^o is increased, P^i will tend to decrease, while π^i usually does not change very much. More precisely, because the cell wall is quite rigid, a plant cell will not change its volume appreciably in response to changes in P^i . (Since the cell wall has elastic properties—cf. Ch. 1—some water flows out as P^i decreases and the cell shrinks, a point which we will return to shortly.) Therefore, if the cell volume can be assumed not to change in response to changes in P^i and no internal solutes leak out, π^i will remain constant. As the external osmotic pressure is raised, π^o will eventually become equal to π^i . In such a plant cell at equilibrium, P^i is zero, which means that no internal hydrostatic pressure is exerted against the cell wall when π^o equals π^i . The cell would thus lose its turgidity. If π^o were increased even further, an appreciable amount of water would flow out of the cell and plasmolysis would occur as the plasma-lemma pulls away from the rigid cell wall. Ignoring for the moment any

overall volume changes of the cell, we find that the condition under which water just begins to move out of the cell—referred to as the point of incipient plasmolysis, illustrated in Figure 3.7a—is

$$\pi^o_{\text{plasmolysis}} = \pi^i \quad (2.19)$$

Equation 2.19 suggests that only a relatively simple measurement ($\pi^o_{\text{plasmolysis}}$) is needed in order to estimate the osmotic pressure (π^i) occurring inside an individual plant cell (see Stadelmann).

The existence of an internal hydrostatic pressure within a plant cell leads to stresses in its cell wall and a resulting elastic deformation or strain, as indicated in Chapter 1. The decrease of P^i to 0 bars—which occurs in taking a turgid plant cell to the point of incipient plasmolysis—must therefore be accompanied by a contraction of the cell as the wall stresses are removed. This decrease in volume means that some water will actually flow out of the cell before the point of incipient plasmolysis is reached. If no internal solute leaves as the cell shrinks, then the osmotic pressure inside will increase (the same amount of solute in a smaller volume). As a useful first approximation, we can assume that the increase in osmotic pressure reciprocally follows the change in volume, i.e., the product of π^i and the cellular volume is approximately constant. With this assumption, the osmotic pressures in cells—described by Equation 2.19, and determined by using the technique of measuring the point of incipient plasmolysis—can be corrected to its original value by using the ratio of the initial volume to the final volume of the cell. The change in volume is only a few percentage points for some plant cells, in which case fairly accurate estimates of π^i can be obtained from plasmolytic studies alone.

Measurements of $\pi^o_{\text{plasmolysis}}$ often give values near 7 bars for cells in storage tissues like onion bulbs or carrot roots and in leaves like those of pea or spinach. These values of the external osmotic pressure provide information on various contributors to the water potential inside the cell, as Equation 2.19 indicates. For the case in which water is in equilibrium within the plant cell at the point of incipient plasmolysis, the term π^i in Equation 2.19 is the osmotic pressure both in the cytoplasm and in the vacuole. Under certain circumstances it is convenient to replace π^i by $\pi_s^i + \tau^i$ (Eq. 2.11), where π_s^i is the osmotic pressure contributed by the internal solutes and τ^i is the matric potential. This relation was invoked for example when the implications of Equation 2.14 ($\pi^{\text{cytoplasm}} = \pi^{\text{vacuole}}$) were discussed, and the various arguments presented at that time extend to the present case. Specifically, since the possible matric potential in the vacuole is probably negligible, $\pi^o_{\text{plasmolysis}}$ should be a rather good estimate

of π_s^{vacuole} . Moreover, $\pi_s^{\text{cytoplasm}}$ is most probably significant, as the water activity in the cytoplasm can be lowered by the many interfaces present. Thus, $\pi_s^{\text{plasmolysis}}$ is an upper limit for $\pi_s^{\text{cytoplasm}}$. We see, therefore, that determination of the external osmotic pressure at the point of incipient plasmolysis provides information on the osmotic pressure existing in different compartments within the plant cell.

Activity and water potential of water vapor. Water molecules in an aqueous solution continually escape into a surrounding gas phase. At the same time, water molecules also condense back into the liquid. The rates of escape and condensation depend *inter alia* on the chemical activities of water in the gas and the liquid phases, the two flows becoming equal at equilibrium. The gas phase adjacent to the given solution then contains as much water as it can hold at that temperature and still remain in equilibrium with the liquid. It is thus *saturated* with water vapor for the particular solution under consideration. The partial pressure exerted by the water vapor in equilibrium with pure water is known as the *saturation vapor pressure*.

The vapor pressure at equilibrium depends on the temperature and the solution but is independent of the relative or absolute amounts of liquid and vapor. When air adjacent to pure water is saturated with water vapor (100% relative humidity), the gas phase then has the maximum water vapor pressure possible at that temperature—unless it is supersaturated, a non-equilibrium situation. This saturation vapor pressure of pure water increases markedly with temperature, e.g., it is equivalent to 4.6 mm of Hg at 0° and 55.3 mm at 40° (see App. II, where the saturation vapor pressure is also expressed in millibars). Thus, air of 100% relative humidity at 0° would be only $(100)(4.6)/(55.3)$, or 8% relative humidity when heated at constant pressure to 40°. As solutes are added to the liquid phase and the activity of its water is thereby lowered, water molecules have less of a tendency to leave the solution, so that the water vapor pressure in the gas phase at equilibrium becomes less—this is one of the colligative properties of solutions that we mentioned earlier. In fact, for dilute solutions the actual partial pressure of water vapor (P_{wv}) at equilibrium depends linearly on the mole fraction of water (N_w) in the liquid phase—this is *Raoult's law*, which is also mentioned in Appendix VII. For pure water, N_w equals one and P_{wv} has its maximum value, viz., P_{wv}^* , the saturation vapor pressure.

The partial pressure of the water vapor determines the activity of water in a gas phase, a_{wv} . If water vapor behaved as an ideal gas, then

$$a_{wv} = \frac{P_{wv}}{P_{wv}^*} = \frac{\% \text{ relative humidity}}{100} \quad (2.20)$$

where P_{wv} is the partial pressure of water vapor in the air under consideration, and P_{wv}^* is the saturation vapor pressure in equilibrium with pure liquid water at the same temperature. To handle deviations from ideality, P_{wv} is replaced by $\gamma_{wv} P_{wv}$, where γ_{wv} is the activity coefficient (more properly, the "fugacity" coefficient) of water vapor. If water vapor obeyed the ideal gas law— $P_j V = n_j RT$, where P_j is the partial pressure of species j in a volume V that contains n_j moles of that gas— γ_{wv} would equal unity. This is an extremely good approximation for most situations of interest in plant physiology. The relative humidity defined by Equation 2.20 is a readily measured quantity reflecting the activity of water in a particular gas phase, and it has been extensively used in studying the water relations of plants.

For water existing as water vapor in air, the chemical potential of the water as defined by Equation 2.4 is simply $\mu_{wv}^* + RT \ln a_{wv} + m_{wv} gh$. (We will also discuss chemical potential in a vapor phase in App. VII.) Using the expression for the activity of water vapor given by Equation 2.20, the water potential of water vapor in a gas phase such as air, Ψ_{wv} , is then

$$\Psi_{wv} = \frac{\mu_{wv} - \mu_{wv}^*}{\bar{V}_w} = \frac{RT}{\bar{V}_w} \ln \left(\frac{\% \text{ relative humidity}}{100} \right) + \rho_w gh \quad (2.21)$$

where a definition of water potential analogous to that given in Equation 2.13 [$\Psi = (\mu_w - \mu_w^*)/\bar{V}_w$] has been used, and $m_{wv}/\bar{V}_w$ (the same as $m_w/\bar{V}_w$) has been replaced by the density of water, ρ_w . Note that $\bar{V}_w$, not $\bar{V}_{wv}$, is used in the definition of Ψ_{wv} in Equation 2.21. This is necessary since the fundamental term representing free energy per mole is the chemical potential—we want to compare $\mu_j - \mu_j^*$ for the two phases when predicting changes at an interface—and thus the proportionality factor between $\mu_w - \mu_w^*$ or $\mu_{wv} - \mu_{wv}^*$ and the more convenient terms, Ψ or Ψ_{wv} , must be the same in each case, viz., $\bar{V}_w$.

Let us next consider the various parameters at the surface of pure water ($a_w = 1$) at atmospheric pressure ($P = 0$ bars) in equilibrium with its vapor. In such a case, P_{wv} equals P_{wv}^* , and we can take the zero level of the gravitational term as the surface of the liquid. Hence, μ_w equals μ_w^* , and μ_{wv} is μ_{wv}^* . Since there is no tendency for a net gain or loss of water molecules from the liquid phase at equilibrium ($\mu_w = \mu_{wv}$), we conclude that the constant μ_w^* must equal the constant μ_{wv}^* when the two phases are at the same temperature.

What happens to P_{wv} as we move vertically upward from pure water at atmospheric pressure in equilibrium with water vapor in the air? If we let h be zero at the surface of the water, Ψ equals zero ($\Psi = P - \pi + \rho_w gh$, Eq. 2.13), and since water vapor is by supposition in equilibrium with the

liquid phase, Ψ_{wv} is also zero. Equation 2.21 indicates that as we go vertically upward in the gas phase, $\rho_w g h$ must make an increasingly positive contribution to Ψ_{wv} . Since we are at equilibrium, the other term in Ψ_{wv} , which equals $(RT/\bar{V}_w) \ln(P_{wv}/P_{wv}^*)$ by Equation 2.20, must make a compensating negative contribution. Thus, at equilibrium P_{wv} must decrease with altitude. We can further appreciate this conclusion simply by noting that gravity attracts the molecules of water vapor and other gases toward the earth, i.e., air pressure is higher at sea level than it is on the top of a mountain. This effect on P_{wv} is generally not very important in plant physiology, since rather great heights are needed before the partial pressure of water vapor is reduced significantly. In particular, let us consider a 7% reduction in P_{wv} from P_{wv}^* (its value at the water surface at equilibrium) to $0.93P_{wv}^*$. Since $RT/\bar{V}_w$ is 1350 bars at 20° (App. III), $(RT/\bar{V}_w) \ln(P_{wv}/P_{wv}^*)$ would change by (1350 bars) $\ln(0.93)$, or -99 bars. As indicated on p. 70, $\rho_w g$ equals 0.098 bar/m, hence a height of (99 bars)/(0.098 bar/m), or 1010 m would be required for the gravitation term to increase by the same amount. In other words, not until we reached a height of 1 km would the partial pressure of water vapor at equilibrium decrease by 7% from its value at sea level, indicating that the gravitational term generally has relatively little influence on Ψ_{wv} over the distances involved for a particular plant.

Plant-air interface. Water equilibrium across the plant-air interface occurs when the water potential in the leaf cells equals that of the surrounding atmosphere. (This presupposes that the leaf and the air are at the same temperature, an aspect that we will reconsider in Ch. 7.) To measure Ψ^{leaf} , the leaf can be placed in a closed chamber and the relative humidity adjusted so that there is no net water gain or loss by the leaf. Such a determination is rather demanding, since small changes in relative humidity have large effects on Ψ_{wv} , as we will now show.

Extremely large negative values are possible for Ψ_{wv}^{air} . In particular, $RT/\bar{V}_w$ at 20° is 1350 bars. In the expression for Ψ_{wv} (Eq. 2.21) this relatively large factor multiplies $\ln(\% \text{ relative humidity}/100)$, and a wide range of relative humidities can occur in the air. By Equation 2.21 and ignoring $\rho_w g h$, a relative humidity of 100% corresponds to a water potential in the vapor phase of (1350 bars) $[\ln(100/100)]$, which equals 0 bars ($\ln 1 = 0$). This Ψ_{wv}^{air} would be in equilibrium with pure water at atmospheric pressure, which also has a water potential of 0 bars. For a relative humidity of 99%, Ψ_{wv}^{air} given by Equation 2.21 equals (1350) $[\ln(99/100)]$, which is -13.5 bars—remember that $\ln(1-x)$ is about $-x$ for $|x| \ll 1$, such as is the value 0.01 in the present case. Hence, going from 100% to 99% relative humidity corresponds to a decrease in water potential of 13.5 bars. Small

changes in relative humidity indeed reflect marked differences in the water potential in air! Finally, we note that a relative humidity of 50% at 20° leads to a Ψ_{wv}^{air} of $(1350) \ln(50/100)$, or -940 bars.

One important consequence of the large negative values of Ψ_{wv}^{air} is easily appreciated by considering values of the water potential that may occur in plant leaves. The actual value of Ψ^{leaf} depends on the ambient conditions as well as the plant type and its physiological status. The range of values for most mesophytes is -3 to -30 bars, with -5 bars being typical for leaves of a garden vegetable such as lettuce. From Equation 2.21, the relative humidity corresponding to a water potential of -5 bars is 99.6%.* Even during a rainstorm the relative humidity of the air rarely exceeds 99%. Since relative humidity is lower than 99.6% under most natural conditions, water is continually being lost from a leaf having an internal water potential of -5 bars. (Again, it should be emphasized that throughout this discussion the leaf is assumed to be at the same temperature as the air—a restriction that we will remove in Ch. 7.) A few desert plants have a Ψ^{leaf} as low as -50 bars. Even in this case of adaptation to arid climates, however, water still tends to leave the plant unless the relative humidity is above 96%.†

Pressure in the cell wall water. Plant cells come into contact with air at the cell walls bounding the intercellular air spaces (cf. Fig. 1.2). Thus, the water potential in the cell walls must be considered with respect to Ψ_{wv} in the adjacent gas phases. The main contributor to Ψ in cell wall water is often the negative hydrostatic pressure arising from surface tension effects at the numerous air-liquid interfaces of the cell wall interstices. In turn, $P^{\text{cell wall}}$ can be related to the geometry of the cell wall pores and the contact angles that occur.

The magnitude of the negative hydrostatic pressure that develops in cell wall water can be analyzed by considering the pressure that occurs in a liquid within a cylindrical pore—the argument being basically the same as the one presented earlier in this chapter in discussing capillary rise. One of the forces acting on the fluid in a narrow pore of radius r is the result of surface tension, σ . This force equals $2\pi r\sigma \cos \alpha$ (cf. Fig. 2.2), where α is the contact angle, a quantity which can be essentially zero for wettable walls. Since the adhesive forces at the wall are transmitted to the body of the fluid by means

* Such an extremely high value for the relative humidity in equilibrium with a Ψ^{leaf} of -5 bars indicates why it is rather difficult to determine Ψ^{leaf} by measuring the Ψ_{wv}^{air} for which no water is gained or lost by the leaf. See Boyer for ways to measure Ψ and Ψ_{wv} .

† Structural modifications are generally more important than a low Ψ^{leaf} for adapting to xerophytic conditions. We will discuss this topic in Chapters 7 and 8.

of its cohesion, a tension or negative hydrostatic pressure develops in the fluid. The total force resisting the surface adhesion can be regarded as this tension times the area over which it acts, πr^2 , hence the force is $\pi r^2 \times (-P)$. Equating the two forces gives the following expression for the pressure developed in the fluid contained within a cylindrical pore:

$$P = -\frac{2\sigma \cos \alpha}{r} \quad (2.22)$$

Adhesion of water at interfaces generally creates negative hydrostatic pressures (such as that described by Eq. 2.22) in the bulk of the fluid. Such a negative P arising from interfacial interactions has sometimes been treated in plant physiology as a positive matric pressure, a convention that we mentioned earlier.

The strong water-wall adhesive forces, which are transmitted throughout the interfibrillar spaces by water-water hydrogen bonding, conceivably can greatly reduce the water potential in the cell wall, as the following calculation indicates. At 20° the surface tension of water is 72.8 dynes/cm or 7.28×10^{-5} bar-cm (App. II). The voids between the fibers in the cell wall are often about 10^{-6} cm (10 nm or 100 Å) across; for wettable walls, $\cos \alpha$ can equal one. For water in such cylindrical pores, Equation 2.22 indicates that P would be $-(2)(7.28 \times 10^{-5} \text{ bar-cm})(1)/(0.5 \times 10^{-6} \text{ cm})$, or -290 bars. This is an estimate of the negative hydrostatic pressure or tension that *could* develop in the aqueous solution within cell wall interstices of typical dimensions, supporting the contention that $\Psi^{\text{cell wall}}$ can be markedly less than zero. Moreover, the previous discussion of the tensile strength of water (p. 52) indicates that the hydrogen bonding in water can withstand such a tension. We shall now consider the actual pressures that develop in the cell wall water.

The relative value of the water potential in the cell wall compared with that in the adjacent vapor phase is of considerable importance in transpiration and for water loss from plant material in general. From Equation 2.21, $\Psi_{\text{ww}}^{\text{air}}$ corresponding to a relative humidity of 95% at 20° is $(1350) \ln(0.95)$, or -69 bars. According to Equation 2.22, a P of -69 bars would occur in a water-containing cylindrical pore with wettable walls having a radius of $-(2)(7.28 \times 10^{-5})(1)/(-69)$, or 2.1×10^{-6} cm, which is 21 nm or 210 Å. In other words, water in 21 nm radius interstices in the cell walls of leaf mesophyll cells would be in equilibrium with air of 95% relative humidity in the intercellular spaces. If the relative humidity near the cell wall surface were decreased, water in such interstices could be lost, but it would still remain in the finer pores where a more negative P or larger tension can

be created. Hence, if the plant material is exposed to air of moderate to low relative humidity—such as can happen for an herbarium specimen—then larger and larger tensions resulting from interfacial interactions develop in the cell walls as the plant material dries out. Water is then retained only in finer and finer pores of the wall, as it must, according to Equation 2.22.

We shall now show the effect of the availability of water adjacent to that in wettable cell walls on the contact angle and on $P^{\text{cell wall}}$. Suppose that pure water in mesophyll cell wall interstices 10 nm across is in equilibrium with water vapor in the intercellular air spaces where the relative humidity is 99%. As we calculated on p. 82, Ψ_{wv} for this relative humidity is -13.5 bars at 20° . Hence, at equilibrium the (pure) water in the cell wall interstices must be under a hydrostatic pressure of -13.5 bars ($\Psi = P - \pi + \rho_w g h$, Eq. 2.13). From Equation 2.22 ($P = -2 \sigma \cos \alpha / r$), and letting σ be 7.28×10^{-5} bar-cm at 20° , we calculate that $\cos \alpha$ must be $-(-13.5 \text{ bars}) / (0.5 \times 10^{-6} \text{ cm}) / [(2)(7.28 \times 10^{-5} \text{ bar-cm})]$, or 0.0464 for the cell wall pores which are 5 nm in radius. When $\cos \alpha$ is 0.0464, α is 87° . For the wettable cell walls, α can be 0° , in which case $\cos \alpha$ is unity and the maximum negative pressure does develop, viz., -290 bars for the pores 5 nm in radius. On the other hand, when water is available, it can be pulled into the interstices by such possible tensions and thereby cause α to increase toward 90° , which leads to a decrease in $\cos \alpha$ and consequently a decrease in the absolute value of the pressure developed. In the present example, α is 87° , P is -13.5 bars, and the pore 5 nm in radius is nearly filled. Thus, the water in the interstices can be nearly flush with the cell wall surface. In fact, the large tensions that could be present in the cell wall generally do not occur in living cells, since water is usually available within the plant and is "pulled" into the interstices, thus nearly filling them, depending of course on their dimensions (cf. Eq. 2.22).

The only contribution to the water potential of the cell wall that we have been considering is the negative pressure that develops because of air-liquid interfaces, which can be the case for pure water. When the gravitational term in Equation 2.13 is ignored, $\Psi^{\text{cell wall}}$ actually equals $P^{\text{cell wall}} - \pi^{\text{cell wall}}$. The bulk solution in the cell wall pores generally has an average osmotic pressure of 3 to 15 bars. The local $\pi^{\text{cell wall}}$ next to the Donnan phase (p. 116) is considerably greater because of the many ions present there; by way of compensation, $P^{\text{cell wall}}$ is also higher near the Donnan phase than in the bulk of the cell wall water.

Water flux. When the water potential inside a cell differs from that outside, the water is no longer in equilibrium and we can expect a water movement toward the region of lower water potential. Our attention here

will be specifically focused on water flow into and out of plant cells. This volume flux of water, J_{V_w} , is assumed to be proportional to the difference in water potential ($\Delta\Psi$) across the membrane or membranes restricting the flow. The proportionality factor indicating the permeability to water flow at the cellular level is expressed by a water conductivity coefficient, L_w :

$$J_{V_w} = L_w \Delta\Psi = L_w(\Psi^0 - \Psi^1) \quad (2.23)$$

In Equation 2.23, J_{V_w} is the volume flow of water per unit area of the barrier per unit time. It can have units of $\text{cm}^3/(\text{cm}^2\text{-sec})$ or cm/sec , which is a velocity. In fact, J_{V_w} is the average velocity of water moving across the barrier being considered.* L_w can be given in $\text{cm}/(\text{sec-bar})$, in which case the water potentials would be expressed in bars. (L_w is usually experimentally the same as L_P , a coefficient also describing water conductivity, which we will formally introduce in Ch. 3 in a rather different manner.)

When Equation 2.23 is applied to cells, Ψ^0 is the water potential in the external solution, and Ψ^1 usually refers to the water potential in the vacuole. L_w then reflects the conductivity for water flow across the cell wall, the plasmalemma, and the tonoplast, all in series. For a group of barriers in series with an overall water conductivity coefficient L_w , $1/L_w$ equals $\sum_j 1/L_{w_j}$, where L_{w_j} is the water conductivity coefficient of barrier j . To see why this is so, let us go back to Equation 2.23, $J_{V_w} = L_w \Delta\Psi$. When the barriers are in series, J_{V_w} is the same across each one, and so J_{V_w} equals $L_{w_j} \Delta\Psi_j$ (also $\Delta\Psi_j = J_{V_w}/L_{w_j}$), where $\Delta\Psi_j$ is the drop in water potential across barrier j , i.e. $\sum_j \Delta\Psi_j = \Delta\Psi$. We therefore obtain the following string of equalities: $J_{V_w}/L_w = \Delta\Psi = \sum_j \Delta\Psi_j = \sum_j J_{V_w}/L_{w_j} = J_{V_w} \sum_j 1/L_{w_j}$; hence, $1/L_w$ must equal $\sum_j 1/L_{w_j}$. (We also note that $1/L_w$ corresponds to a resistance and the resistance of a group of resistors in series is the sum of the resistances, $\sum_j 1/L_{w_j}$.)

We shall now consider a membrane separating two solutions that differ only in osmotic pressure. This will help us to relate L_w to the permeability

* To help see why this is so, consider a volume element $A \text{ cm}^2$ in cross-sectional area, extending back from the barrier for a length equal to the average water velocity, $\bar{v}_w$, multiplied by dt . In time dt this volume element ($A\bar{v}_w dt$) would cross area A of the barrier, and for such a period the volume flow of water per unit area (J_{V_w}) would be $(A\bar{v}_w dt)/(A dt)$, which is $\bar{v}_w$.

coefficient of water, P_w , and also to view Fick's first law [$J_j = P_j(c_j^o - c_j^i)$, Eq. 1.8] in a slightly different way. The proper form for Fick's first law when describing the diffusion of the solvent water is $J_{V_w} = P_w(N_w^o - N_w^i)$. By Equation 2.23, J_{V_w} also equals $L_w(\Psi^o - \Psi^i)$, which becomes $L_w(\pi^i - \pi^o)$ in our case (recall that $\Psi = P - \pi + \rho_w gh$, Eq. 2.13). Thus, a flux of water toward a region of higher osmotic pressure (Eq. 2.23) is equivalent to J_{V_w} toward a region where water is less concentrated in the sense of having a lower mole fraction (Fick's first law applied to water). Let us pursue this one step further. Using Equations 2.8 through 2.10 we can obtain the following string of equalities: $N_w \cong 1 - \sum_j n_j/n_w \cong 1 - \bar{V}_w \pi_s/RT$. There-

fore, $N_w^o - N_w^i$ equals $(1 - \bar{V}_w \pi_s^o/RT) - (1 - \bar{V}_w \pi_s^i/RT)$, or $\bar{V}_w(\pi_s^i - \pi_s^o)/RT$. When we incorporate this last relation into our two expressions for the volume flux of water, we obtain $J_{V_w} = L_w(\pi^i - \pi^o) = P_w(N_w^o - N_w^i) = P_w \bar{V}_w(\pi_s^i - \pi_s^o)/RT$ (π^i is here the same as π_s^i). Hence, L_w equals $P_w \bar{V}_w/RT$, which states that the water conductivity coefficient (L_w) is proportional to the water permeability coefficient (P_w). Next, we will estimate a possible value for L_w . P_w is generally at most 10^{-2} cm/sec for plasmalemmas (p. 32), RT is 24,370 cm³-bars/mole at 20° (App. III), and $\bar{V}_w$ is 18 cm³/mole. Hence, L_w might be $(10^{-2} \text{ cm/sec})(18 \text{ cm}^3/\text{mole})/(24,370 \text{ cm}^3\text{-bars/mole})$, or 0.74×10^{-5} cm/(sec-bar). When P_w is less than 10^{-2} cm/sec, L_w will be proportionally less than 0.74×10^{-5} cm/(sec-bar).

When the value of the water conductivity coefficient is known, the water potential difference necessary to give an observed water flux can be calculated by using Equation 2.23. For the internodal cells of *Nitella* and *Chara*, L_w for water entry can be about 10^{-5} cm/(sec-bar)—a large value for L_w , see Dainty. For convenience of calculation, we will consider cylindrical cells 10 cm long and 1 mm in diameter as an approximate model for such algal cells (cf. Fig. 3.4). The surface area across which the water flux occurs is $(2\pi r)l$, where r is the cell radius (0.05 cm) and l is the cell length (10 cm). Thus, the area equals $(2\pi)(0.05 \text{ cm})(10 \text{ cm})$, or $\pi \text{ cm}^2$. (The area of each end of the cylinder (πr^2) is much less than $2\pi rl$; in any case, a water flux from the external solution across them would not be expected, since they are in contact with other cells, not the bathing solution—cf. Fig. 3.4.) The volume of a cylinder is $\pi r^2 l$, which equals $(\pi)(0.05 \text{ cm})^2(10 \text{ cm})$, or $\pi/40 \text{ cm}^3$ in the present case. Internodal cells of *Nitella* and *Chara* grow relatively slowly—a change in volume of about 1% per day being a possible growth rate for the fairly mature cells which were used in determining L_w . This growth rate means that the water content increases by about 1% of the volume per day. The total volume flux of water into the above cell is therefore (0.01)

$(\pi/40) \text{ cm}^3/\text{day}$. Such an inflow of water per day is equivalent to $[(\pi/4000) \text{ cm}^3/\text{day}](1/24 \text{ day/hr})(1/3600 \text{ hr/sec})$, or $3\pi \times 10^{-9} \text{ cm}^3$ of water moving into a cell/sec. This water influx occurs across a surface area of $\pi \text{ cm}^2$ per cell, as we just calculated, hence the rate of volume flow of water per cm^2 is $(3\pi \times 10^{-9} \text{ cm}^3/\text{sec})/(\pi \text{ cm}^2)$, or $3 \times 10^{-9} \text{ cm}^3/(\text{cm}^2 - \text{sec})$. But such a quantity is precisely what is meant by J_{V_w} in Equation 2.23. Using the appropriate value of L_w for these internodal cells, $10^{-5} \text{ cm/sec-bar}$, Equation 2.23 indicates that $\Delta\Psi$ is $(3 \times 10^{-9} \text{ cm/sec})/(10^{-5} \text{ cm/sec-bar})$ or $3 \times 10^{-4} \text{ bar}$. In other words, the internal water potential (Ψ^i) needed to sustain the water influx accompanying a growth of about 1% per day is $3 \times 10^{-4} \text{ bar}$ less than the outside water potential (Ψ^o). *Nitella* and *Chara* can grow in pond water, which is a dilute aqueous solution often having a water potential near -0.07 bar . Thus, Ψ^i need be only slightly more negative than -0.07 bar to account for the influx of water accompanying a growth of 1% per day.

Problems

2.1 The rise of water in a long capillary 2 mm in diameter and with wettable walls is 1.5 cm. (a) If the capillary is tilted 45° from the horizontal, what is the vertical height of the rise? (b) If sucrose is added to the solution so that the density becomes 1.2 g/cm^3 , what is the height of the rise? (c) If the capillary extends only 0.75 cm above the main surface of the water, what is the contact angle in the capillary? (d) If the wall of the capillary is treated so that the contact angle becomes 60° , what is the height of the rise? (e) What is the rise of water in a capillary similar to the original one, but with a $1 \mu\text{m}$ radius? (f) In which of the 5 cases is the greatest weight supported by capillary (surface tension) forces?

2.2 A neutral solute at equilibrium has a concentration of 0.1 molal on one side of a barrier permeable only to water and a c_{solute} of 1 molal on the other side. Let the partial molal volume of the solute be $40 \text{ cm}^3/\text{mole}$, the temperature be 20° , and the activity coefficients of the solute and the water be unity, except in (c). (a) What is the hydrostatic pressure difference (in bars) across the barrier? (b) What would be your answer to (a), if the barrier were permeable to solute only? (c) If γ_{solute} is 0.5 on the more concentrated side and if other conditions are unchanged, what is ΔP across the barrier permeable to the solute only? (d) What would happen at equilibrium if the barrier were permeable to both water and solute? (e) If P on the 0.1 molal side is the same as atmospheric pressure, what is the chemical potential of the solute there?

2.3 An ideal solution contains 80 g of sorbitol (molecular weight of 182 g/mole) in 1000 g of water at 20° . (a) What are N_w and a_w in the solution? (b) What is the osmotic pressure of the sorbitol solution? (c) By what percent is the activity

of water reduced for an osmotic pressure of 10 bars at 20° compared with the value of a_w for pure water? (d) If we assume that activity coefficients are unity, what concentration of a solute corresponds to a π of 10 bars at 20° ? (e) An 0.25 mM solution of a particular polymer has a measured osmotic pressure at 20° of 0.1 bar. What is the osmotic pressure predicted by the Van't Hoff relation (Eq. 2.10)? Explain any discrepancies. (f) In the vacuole of a certain cell the mole fraction of water is 0.98, the hydrostatic pressure is 8.0 bars, and the temperature is 20° . Assuming activity coefficients are unity, what is the water potential in the vacuole?

2.4 A tank 10 m tall is filled with an ideal solution at 20° . We will assume that the system is in equilibrium and that the zero level for the gravitational term is at the top of the tank where Ψ is -6.00 bars. (a) What is the water potential 10 cm below the surface and at the bottom of the tank? (b) What is π at the upper surface, 10 cm below the surface, and at the bottom of the tank? (c) What is P and the gravitational term at the three levels in (b)? (d) What is the relative humidity that would be in equilibrium with the water in the tank?

2.5 Chloroplasts are isolated from a plant cell whose cytoplasm has an osmotic pressure of 4 bars at 20° . When the chloroplasts are placed in solutions at 20° containing an impermeable solute, the volumes are $36 \mu\text{m}^3$ at an external osmotic pressure of $3\frac{1}{3}$ bars, $28 \mu\text{m}^3$ at 5 bars, and $20 \mu\text{m}^3$ at 10 bars. Assume that activity coefficients are unity. (a) What is the volume of the chloroplasts in the plant cell? (b) What is the nonaqueous volume per chloroplast? (c) What volume fraction of the chloroplast is occupied by water *in vivo*? (d) What is the content of osmotically active particles per chloroplast?

2.6 A cell has a water conductivity coefficient (L_w) equal to 10^{-5} cm/sec-bar. Let the internal osmotic pressure be 10 bars and the internal hydrostatic pressure be 6 bars at 20° . (a) What is the net volume flux of water into or out of the cell when it is placed in pure water at atmospheric pressure? (b) What is the water flux at the point of incipient plasmolysis? (c) What is the water flux when the external solution is in equilibrium with a gas phase at 97% relative humidity? (d) Assume that the water in the cell walls is in equilibrium with the internal cellular water. What are $\cos \alpha$ and the contact angle at the air-water interface for cylindrical cell wall pores that are 20 nm in diameter?

References

- Adamson, A. W. 1967. *Physical Chemistry of Surfaces*, 2nd ed., Interscience (Wiley), New York.
- Bikerman, J. J. 1970. *Physical Surfaces*, Academic Press, New York.
- Boyer, J. S. 1969. Measurement of the water status of plants. *Annual Review of Plant Physiology* **20**: 351-364.
- Bull, H. B. 1964. *An Introduction to Physical Biochemistry*, F. A. Davis, Philadelphia.

- Dainty, J. 1963. Water relations of plant cells. *Advances in Botanical Research* 1: 279-326.
- Davies, J. T., and E. K. Rideal. 1963. *Interfacial Phenomena*, 2nd ed., Academic Press, New York.
- Dick, D. A. T. 1966. *Cell Water*, Butterworths, London.
- Edsall, J. T., and J. Wyman. 1958. *Biophysical Chemistry*, Vol. I, Academic Press, New York.
- Eisenberg, D., and W. Kauzmann. 1969. *The Structure and Properties of Water*, Oxford University Press, Oxford.
- Giese, A. C. 1973. *Cell Physiology*, 4th ed., Saunders, Philadelphia.
- Hayward, A. T. J. 1971. Negative pressure in liquids: Can it be harnessed to serve man? *American Scientist* 59: 434-443.
- Lin, D. C., and P. S. Nobel. 1971. Control of photosynthesis by Mg^{2+} . *Archives of Biochemistry and Biophysics* 145: 622-632.
- Morris, J. G. 1968. *A Biologist's Physical Chemistry*, Addison-Wesley, Reading (Mass.).
- Nobel, P. S. 1969. The Boyle-Van't Hoff relation. *Journal of Theoretical Biology* 23: 375-379.
- Pauling, L. 1964. *College Chemistry*, 3rd ed., W. H. Freeman and Company, San Francisco.
- Salisbury, F. B., and C. Ross. 1969. *Plant Physiology*, Wadsworth, Belmont (Calif.).
- Slatyer, R. O. 1967. *Plant-Water Relationships*, Academic Press, New York.
- Stadelmann, E. J. 1966. Evaluation of turgidity, plasmolysis, and deplasmolysis of plant cells, in *Methods in Cell Physiology*, Vol. II, D. M. Prescott, ed., Academic Press, New York, pp. 143-216.
- van Holde, K. E. 1971. *Physical Biochemistry*, Prentice-Hall, Englewood Cliffs (N.J.).
- Zimmermann, M. H., and C. L. Brown. 1971. *Trees: Structure and Function*, Springer Verlag, New York.

CHAPTER THREE

SOLUTES

In this chapter we will turn our attention to the properties of solutes, especially in regard to their crossing of membranes. We will compare the chemical potentials in the aqueous phases on the two sides of a membrane or other region in order to predict the direction of the passive solute fluxes as well as the driving forces leading to such motion. Moreover, we will show how the fluxes of charged species can account for the electrical potential differences observed across biological membranes.

Many of the solute properties important in plant physiology are intertwined with those of the ubiquitous solvent, water. For example, the osmotic pressure term in the chemical potential of water is mainly due to the decrease of the water activity caused by the solutes ($RT \ln a_w = -\bar{V}_w \pi$ by Eq. 2.7). Also, the movement of water through the soil to the root and then to the xylem can influence the entry of dissolved nutrients, while the subsequent distribution of these nutrients throughout the plant depends on water movement in the xylem (and the phloem in some cases). However, in contrast with water, solute molecules can carry a net positive or negative electrical charge. For such charged particles, the electrical terms must be included in the chemical potentials, which leads to a consideration of electrical phenomena in general and an interpretation of the electrical potential drops present across biological membranes in particular. Whether an observed ionic flux of some species into or out of a cell can be accounted for by the passive process of diffusion depends on the differences in both the concentration of that species and the electrical potential between the inside and the outside. Ions can also be actively transported across a membrane, in which case metabolic energy is involved in some way in moving the solutes.

When both solutes and water traverse the same barrier we should replace the classical thermodynamic approach by a description based on irreversible

thermodynamics. The various forces and fluxes are then viewed as interacting, which means that the movement of water across a membrane influences the movement of solutes, and vice versa. This more general approach helps us quantitatively describe the observation that the osmotic pressure difference *effective* in causing a volume flux across a membrane that is permeable to both water and solutes is less than the *actual* osmotic pressure difference across that membrane.

Chemical potential of ions

We have already introduced the concept of chemical potential in Chapter 2. Since a substance spontaneously tends to move to regions where its chemical potential is lower, this quantity is a useful parameter for analyzing passive movements of solutes. Using a linear combination of the various contributors, the chemical potential of any species j is given by Equation 2.4 as follows: $\mu_j = \mu_j^* + RT \ln a_j + \bar{V}_j P + z_j FE + m_j gh$. Since water is uncharged ($z_w = 0$), the electrical term does not enter into its chemical potential. For ions, however, $z_j FE$ becomes important. In fact, for charged solutes under most conditions of biological interest, differences in the electrical term are usually far larger than are changes in the terms for the hydrostatic pressure or gravity. In particular, for movements across membranes, Δh is zero and so we will omit the gravitational term in this chapter, while changes in the $\bar{V}_j P$ term are also generally ignored because they are relatively small (as we will indicate below). Therefore the chemical potential generally used when dealing with ions, $\mu_j^* + RT \ln a_j + z_j FE$, is commonly referred to as the *electrochemical* potential. Although this emphasizes the role played by electrical potentials, “chemical potential” is only a shorthand expression for the sum of *all* the different contributors affecting a particular species, and it is not really necessary to use a special term like “electrochemical” potential when dealing with ions.

Electrical potential. The difference in electrical potential E between two locations is a measure of the amount of work involved in moving a charge from one location to the other. When the charge is expressed in coulombs and the potential difference in volts, then the amount of work in joules equals the product of the two. The zero level for electrical potential is actually arbitrary. Since both the initial and the final electrical potential must be expressed relative to the same zero level, the arbitrariness is of no consequence when the electrical potential difference (final minus initial potential) is determined.

The charge carried by an ion of species j is a positive or negative integer z_j (the charge *number*) times the charge on a proton. For instance, z_j is $+1$ for the potassium ion and -2 for sulfate (SO_4^{--}). The electrical charge carried by a proton is commonly called the *electronic charge*, since it is the same in magnitude as the charge on an electron (but opposite in sign, of course). One unit for charge is the coulomb; a proton has a charge of 1.6021×10^{-19} coulomb. Thus, a mole (Avogadro's number) of protons would have a charge equal to $(6.02252 \times 10^{23} \text{ protons/mole}) (1.6021 \times 10^{-19} \text{ coulomb/proton})$, or 96,487 coulombs/mole. Such a unit consisting of Avogadro's number of electronic charges (i.e., one mole of single, positive charges) is called the Faraday, F . This quantity appearing in the electrical term of the chemical potential (cf. Eq. 2.4) equals 96,487 coulombs/mole or 96,487 joules/mole-volt—a coulomb-volt is a joule—which can also be expressed as 23,060 cal/mole-volt (see App. II).

To illustrate the rather large contribution the electrical term can make to differences in the chemical potential of a charged substance compared with the effect of changes in hydrostatic pressure, let us consider a small monovalent cation ($z_j = +1$) that has a partial molal volume ($\bar{V}_j$) equal to $30 \text{ cm}^3/\text{mole}$. What electrical potential difference (ΔE) in volts (v) corresponds to the same contribution to the chemical potential of such an ion as a hydrostatic pressure drop (ΔP) of one bar? In other words, what is ΔE so that $z_j F \Delta E$ equals $\bar{V}_j \Delta P$ under these conditions? One bar is 10^6 dynes/cm^2 , or 0.1 joule/cm^3 (App. III). Thus, ΔE need be only $(30 \text{ cm}^3/\text{mole})(0.1 \text{ joule/cm}^3)/[(1)(96,487 \text{ joules/mole-volt})]$, that is, $3.1 \times 10^{-5} \text{ v}$ (0.031 mv). This is an extremely small electrical potential difference! For comparison, the drop in electrical potential across a biological membrane is often near 100 mv, which is $(100)/(0.031)$ or 3200 times larger than the potential difference calculated here. Stated another way, 100 mv across a membrane would make the same contribution to the chemical potential difference as a drop of 3200 bars of hydrostatic pressure from one side of the barrier to the other for the above case of a monovalent cation having a $\bar{V}_j$ of $30 \text{ cm}^3/\text{mole}$. Most hydrostatic pressures encountered in plant or cell physiology are only a few bars. Thus, hydrostatic pressure drops across membranes are usually negligible in comparison with the electrical contributions to the chemical potential differences of ions from one side of the membranes to the other.

Electroneutrality and membrane capacitance. Another consequence of the relatively large magnitude of electrical effects is the general occurrence of *electroneutrality*. Thus, in most aqueous regions that are large compared with atomic dimensions the total electrical charge carried by the cations is essen-

tially equal in magnitude to that carried by the anions. What is the situation for plant cells? Do we ever have an excess of negative or positive charges in cells or organelles? If there were a net charge in some region, such as near a membrane, an electrical potential difference would exist from one part of the region to another. Can we relate the size of the electrical potential drop to the net charge?

In order to relate charges and ΔE s, we need to introduce a new term, *capacitance*. Electrical capacitance is simply a coefficient of proportionality between a net charge and the resulting electrical potential difference. A high capacitance means that the region has the *capacity* to have many uncompensated charges without at the same time developing a large electrical potential drop. Let us now express this symbolically. The magnitude of such an electrical potential difference, ΔE , is related to the capacitance of the region (C) as follows: *

$$Q = C\Delta E \quad (3.1)$$

where Q is the net charge. The unit for capacitance is the *farad* (f), which equals 1 coulomb/volt. The capacitances of most biological membranes are approximately the same per unit area, about 1 microfarad (μf)/ cm^2 (see Bull, Cole, Keynes in Agin, or Ruch and Patton).

For convenience in estimating the net charge inside a cell which would lead to a typical membrane potential, we will consider a spherical cell of radius r . Suppose that the uncanceled or net charges are uniformly distributed in space at a concentration, c . (For a conductor—a body in which electrical charges can freely move—such as an aqueous solution, the uncanceled or net charges would not remain uniformly distributed. Rather, they repel each other and would therefore collect at the inner surface of the sphere. The quantity c is therefore the hypothetical concentration of the net charge if it were uniformly distributed throughout the interior of the sphere.) The charge Q within the sphere of radius r is then $(4/3)\pi r^3 cF$. (The concentration, c , can be in moles/ cm^3 , and here refers to the net concentration of uncompensated singly charged particles; F , which is in coulombs/mole, is necessary to convert from concentration to electrical charge.) The capacitance C of the sphere is $4\pi r^2 C'$, where $4\pi r^2$ is the surface area of a sphere and C' is the capacitance per unit area. After substituting these values of Q and C into Equation 3.1 and rearranging, we obtain the

* In keeping with most biological literature, E in electrical equations throughout this book refers to electrical potentials, not electric field intensities, as in many physics texts.

following expression for the electrical potential difference in the case of a spherical capacitor:

$$\Delta E = \frac{rcF}{3C'} \quad (3.2)$$

Equation 3.2 gives the electrical potential difference from the center of the sphere to just outside its surface. For a conductor, the internal uncompensated charges are found near the surface. In that case, ΔE actually occurs across the bounding surface (such as a membrane) surrounding the spherical body under consideration. Equation 3.2 indicates that the electrical potential difference developed is directly proportional to the average concentration of net charge enclosed, and inversely proportional to the capacitance per unit area of the sphere.

To apply Equation 3.2 to a specific situation, let us take a spherical cell with a radius of $30\ \mu\text{m}$ and an electrical potential difference across the membrane (inside negative) of $-100\ \text{mv}$, a value close to that occurring for many cells. If the membrane capacitance per unit area has a typical value of $1\ \mu\text{f}/\text{cm}^2$, to what net charge concentration in the cell does this potential drop correspond? Since a farad is a coulomb/volt, one $\mu\text{f}/\text{cm}^2$ is $10^{-6}\ \text{coulomb}/\text{v}\cdot\text{cm}^2$ while F equals $96,487\ \text{coulombs}/\text{mole}$ (Apps. II and III). Using Equation 3.2, c equals $3C'\Delta E/rF$, hence the average concentration of net charge would be $(3)(10^{-6}\ \text{coulomb}/\text{v}\cdot\text{cm}^2)(-0.1\ \text{v})/[(30 \times 10^{-4}\ \text{cm})(96,487\ \text{coulombs}/\text{mole})]$, or $-1.0 \times 10^{-9}\ \text{mole}/\text{cm}^3$, which is $-1.0 \times 10^{-6}\ \text{mole}/\text{liter}$ ($-1\ \mu\text{M}$). The sign of the charge is negative, indicating more internal anions than cations. The average concentration of the net (uncanceled) charge leading to a considerable electrical potential difference is rather small, an important point that we will consider next.

It is instructive to compare the net charge concentration averaged over the volume of the cell (c in Eq. 3.2) with the total concentration of anions and cations in a plant cell. Specifically, since the positive and negative ions in plant cells can each have a total concentration near $0.1\ \text{M}$, the above calculated excess of $-1\ \mu\text{M}$ is only about one extra negative charge per $(0.1\ \text{M})/(10^{-6}\ \text{M})$, or 10^5 anions. Expressed another way, the total charge of the cations inside such a cell equals or compensates that of the anions to within about one part in 100,000. When cations are taken up by a cell to any appreciable extent, either anions must accompany them and/or cations must be released from inside the cell. Otherwise, if marked departures from electrical neutrality were allowed to occur in some region, sizable electrical potential differences would build up.

Activity coefficients of ions. We will now turn our attention from the

electrical term in the chemical potential to the activity one, specifically to the chemical activity itself. As indicated in Chapter 2, the activity of species j , a_j , is the thermodynamically effective concentration. For charged particles in an aqueous solution this activity can differ appreciably from the actual concentration, c_j —a point that has not always been adequately recognized in dealing with ions. By Equation 2.5, a_j equals $\gamma_j c_j$, where γ_j is the activity coefficient of species j .

A quantitative description of the dependence of the activity coefficients of ions on the concentration of the various species in the solution was developed by Debye and Hückel in the 1920s (see Bull, Edsall and Wyman, MacInnes, or Robinson and Stokes for further details on the Debye-Hückel theory). In a local region around a particular ion, the electrostatic forces describable by relations such as Equation 2.3 constrain the movement of other ions. As the concentration increases, the average distance between the ions becomes less, which facilitates ion-ion interactions. Equation 2.3 [electrical force = $Q_1 Q_2 / (4\pi\epsilon_0 D r^2)$] indicates, for example, that the electrostatic interaction between two charged particles varies inversely as the square of their separation, thus, electrical forces increase greatly as the ions get closer and closer together. When ions of opposite sign attract each other, the various other interactions of both ions are restricted, thus lowering their thermodynamically effective concentration or activity.

A simple and approximate form of the Debye-Hückel equation appropriate for estimating the values of activity coefficients of ions in relatively dilute aqueous solutions at 25° is

$$\log \gamma_{\pm} = \frac{0.509 z_+ z_- \sqrt{\frac{1}{2} \sum_j c_j z_j^2}}{1 + \sqrt{\frac{1}{2} \sum_j c_j z_j^2}} \quad (3.3)$$

where z_+ is the charge number of the cation, z_- that of the anion, concentrations are expressed in molarities, and the summations are over all charged species. Thermodynamic measurements cannot give the activity coefficient of a single ion, because we cannot have a solution of one type of ion by itself in which to measure γ_+ or γ_- . In fact, activity coefficients of ions always occur as the products of those of cations and anions, hence $\gamma_{\pm}$ in Equation 3.3 represents the mean activity coefficient of some cation-anion pair having charge numbers z_+ and z_- . Since z_- is negative, Equation 3.3 indicates that $\log \gamma_{\pm}$ is also negative, and $\gamma_{\pm}$ is less than one. The activities of ions in dilute aqueous solutions are thus less than their concentrations, as we would indeed expect.

To estimate $\gamma_{\pm}$ under conditions approximating those that might occur in a plant or animal cell, let us consider an aqueous solution containing 0.100 M of both monovalent cations and anions and 0.025 M of both divalent cations and anions. For this solution, $\frac{1}{2} \sum_j c_j z_j^2$ (known as the *ionic strength*) is equal to $\frac{1}{2}[(0.100)(1)^2 + (0.100)(-1)^2 + (0.025)(2)^2 + (0.025)(-2)^2]$, or 0.200 M. From Equation 3.3, $\log \gamma_{\pm}$ is then $(0.509)(1)(-1)(\sqrt{0.200})/(1 + \sqrt{0.200})$, or -0.157 for the monovalent ions. This corresponds to a mean activity coefficient of only 0.70, a value considerably less than one.

The activity coefficient of a particular ionic species depends on all the ions in the solution, as is indicated by the ionic strength terms in Equation 3.3. Therefore, even when some particular ionic species is itself dilute, its activity coefficient can still be appreciably less than one because of the many electrostatic interactions with other ions. The departure from 1.00 for activity coefficients is even greater for divalent and trivalent ions than for monovalent ions, as the $z_+ z_-$ factor in Equation 3.3 indicates. Thus although activity coefficients of ions are often set equal to unity for convenience, this is obviously not always justified. A practical difficulty arising under most experimental situations is that c_j is much easier to determine than a_j , especially for compartments like the cytoplasm or the interior of a chloroplast. In those circumstances where a_j has been replaced by c_j , caution must be exercised in the interpretations or conclusions. The activity coefficients for nonelectrolytes and water are generally much closer to unity than are those for ions, hence the assumption involved in replacing a_j by c_j for such neutral species is not as severe as for the charged substances (e.g., γ_{sucrose} is about 0.96 for 0.3 M sucrose). An all-inclusive theory for activity coefficients is rather complicated and beyond the scope of this text. Equation 3.3 is presented only to illustrate in a quantitative manner that activity coefficients of ions can be appreciably less than 1.00 under conditions that may occur in a plant cell.

Nernst potential. Having considered the electrical and activity terms in some detail, we should now turn to the role of these quantities in the chemical potential of ions. Specifically, we will consider the deceptively simple and yet extremely important relationship between the electrical potential difference across a membrane and the accompanying distribution of ions across it at equilibrium.

When ions of some species j are in equilibrium across a membrane, its chemical potential outside (o) is the same as inside (i), i.e., $\mu_j^o = \mu_j^i$. A difference in the hydrostatic pressure term almost always makes a negligible contribution to the chemical potential differences of ions across membranes, therefore $\bar{V}_j P$ can be omitted from μ_j in the present case. With this approxi-

mation and the definition of chemical potential (Eq. 2.4 without the gravitational term), the condition for equilibrium of ionic species j across the membrane is given by:

$$\mu_j^* + RT \ln a_j^o + z_j F E^o = \mu_j^* + RT \ln a_j^i + z_j F E^i \quad (3.4)$$

The term μ_j^* in Equation 3.4 is a constant referring to the same standard state of species j in the aqueous solutions on both sides of the membrane, and can be canceled from the equation.

Upon solving Equation 3.4 for the electrical potential difference, $E^i - E^o$, across the membrane *at equilibrium*, we obtain the following important relationship:

$$\begin{aligned} E_{N_j} = E^i - E^o &= \frac{RT}{z_j F} \ln \frac{a_j^o}{a_j^i} \\ &= \frac{59.2}{z_j} \log \frac{a_j^o}{a_j^i} \text{ mv at } 25^\circ \end{aligned} \quad (3.5)$$

The electrical potential difference E_{N_j} in Equation 3.5 is called the *Nernst potential* of species j , i.e., $E^i - E^o = \Delta E = E_{N_j}$ in this case, where the subscript N stands for Nernst, who first derived this relation about 1900.* We derived it by assuming equality of the chemical potentials of some charged species on two sides of a membrane, but the Nernst potential can be considered in a broader context (discussed below). For convenience of calculation, we can replace the natural logarithm ($\ln$) by $2.303 \log$, where $\log$ is the common logarithm to the base 10 (see Apps. III, IV, and V). The quantity $2.303 RT/F$ equals 58.2 mv at 20° , 59.2 mv at 25° (cf. Eq. 3.5), and 60.2 mv at 30° (App. III). Using such numerical values, we can express the Nernst potential in the useful form presented in the second line of Equation 3.5.

Equation 3.5, the Nernst equation, is an equilibrium statement showing how the internal and external activities of ionic species j are related to the electrical potential difference across the membrane (cf. Fig. 3.1). At equilibrium, a tenfold difference in activity of a monovalent ion across some membrane is energetically equivalent to a 59 mv difference in electrical potential (at 25°). Hence, a relatively small electrical potential drop can balance a large difference in activity across a membrane. For some calculations, γ_j^o/γ_j^i is set equal to one (a less stringent assumption than setting both γ_j^o and γ_j^i equal to one). Under this condition, a_j^o/a_j^i in Equation 3.5

* Nernst made many important contributions to the understanding of the physical

becomes the ratio of the concentrations, c_j^o/c_j^i ($a_j = \gamma_j c_j$, Eq. 2.5). Such a substitution may be justified when the ionic strengths on the two sides of the membrane are approximately the same, but it can lead to errors when the outside solution is much more dilute than the internal one, as would occur for *Chara* or *Nitella* in pond water.

Throughout the rest of this book we will represent the *actual* electrical potential drop existing across a membrane, $E^i - E^o$, by E_M , where the subscript M refers to membrane. Hence, for both equilibrium and non-equilibrium situations, we have $E^i - E^o = \Delta E = E_M$. When a particular ionic species j is in equilibrium across some membrane, E_M equals E_{N_j} , the Nernst potential for that species. However, regardless of the actual electrical potential difference existing across a membrane (E_M), a Nernst potential for an individual ionic species j can always be *calculated* from Equation 3.5 by using the ratio of the outside to the inside activity (a_j^o/a_j^i) or concentration (c_j^o/c_j^i), e.g., $E_{N_j} = (RT/z_j F) \ln (c_j^o/c_j^i)$. For many plant cells, the Nernst potential across the plasmalemma or the tonoplast calculated for potassium ions is close to the electrical potential differences measured across the membranes. If some ionic species cannot penetrate the membrane or is actively transported across it, E_{N_j} can markedly differ from E_M . In fact, the minimum amount of energy needed to transport ions across a membrane is proportional to the difference between E_{N_j} and E_M (see Eq. 3.26). The aqueous phases designated as inside and outside can have more than one membrane intervening between them. For instance, the vacuolar sap and an external solution—the regions often considered experimentally—have two membranes separating them, the plasmalemma and the tonoplast. The thermodynamic arguments remain the same in the case of multiple membranes, with E_M and E_{N_j} referring to the electrical potential differences between the two regions actually under consideration, regardless of how many membranes occur between them.

Example of E_{N_K} . Data obtained on potassium for the large internodal cells of the green freshwater alga *Chara australis* are convenient for illustrating the use of the Nernst equation (see Vorobiev). The potassium ion activity in the medium bathing the cells, a_K^o (where the element symbol K as a subscript is the conventional shorthand notation for K^+) was 0.096 mM, while a_K^i in the vacuole was measured as 48 mM. (See App. I for a comment on the units of activity.) Thus, a_K^o/a_K^i was (0.096 mM)/(48 mM), or 1/500. Using Equation 3.5 (with a factor of 58.2, since measurement was at 20°), the Nernst potential for potassium is (58.2 mv) log (1/500), or -157 mv. The measured electrical potential of the vacuole relative to the external solution, E_M , was -155 mv—very close to the calculated Nernst potential for potassium. Thus, potassium in this case may be

in equilibrium between the external solution and the vacuole. The potassium concentration in the vacuole of these *Chara* cells was 60 mM. The activity coefficient for potassium in the vacuole (γ_K^i) equals a_K^i/c_K^i ($a_j = \gamma_j c_j$, Eq. 2.5), therefore γ_K^i was (48 mM)/(60 mM), or 0.80, while the potassium activity coefficient in the bathing solution (γ_K^o) was about 0.96. For this example, where there are large differences in the internal and the external concentrations, the ratio γ_K^o/γ_K^i is (0.96)/(0.80), or 1.20, which differs appreciably from 1.00. If instead of activities concentrations had been used in Equation 3.5 ($c_K^o/c_K^i = 0.1/60 = 1/600$), E_{N_K} would have been calculated as -162 mv, which is somewhat lower than the measured potential of -155 mv. Calculating from the concentration ratio, the suggestion that potassium is in equilibrium from the bathing solution to the vacuole could not have been made with much certainty, if at all.

Equilibrium does not require that the various forces acting on a substance are zero, rather, that they cancel each other. In the above example for *Chara* the factors that tend to cause potassium to move are the differences both in its activity (or concentration) and the electrical potential across the membranes. The activity of potassium ions was much higher in the vacuole than in the external solution (cf. Fig. 3.1). The activity term in the chemical potential therefore represents a driving force on potassium directed from the inside of the cell to the bathing solution. The electrical potential is lower inside the cell as is indicated in Figure 3.1, hence the electrical driving force on the positively charged potassium ions tends to cause potassium entry into the cell. At equilibrium these two tendencies for movement in opposite directions are balanced and no net flux of potassium occurs. As indicated above, the electrical potential difference existing across a membrane when K^+ is in equilibrium is the Nernst potential of potassium, E_{N_K} . Generally, c_K^i and a_K^i for both plant and animal cells are much higher than c_K^o and a_K^o , and potassium is often close to equilibrium across the cellular membranes. From these observations, we can expect that the interiors of cells are usually at negative electrical potentials compared with the outside solutions, as is indeed the case. In general, the chemical potentials of ions are not equal in all regions of interest, hence passive movements toward lower μ_j occur—a topic we will turn to next.

Fluxes and diffusion potentials

Fluxes of many different solutes are continually occurring across biological membranes. Influxes take mineral nutrients into a growing cell while certain

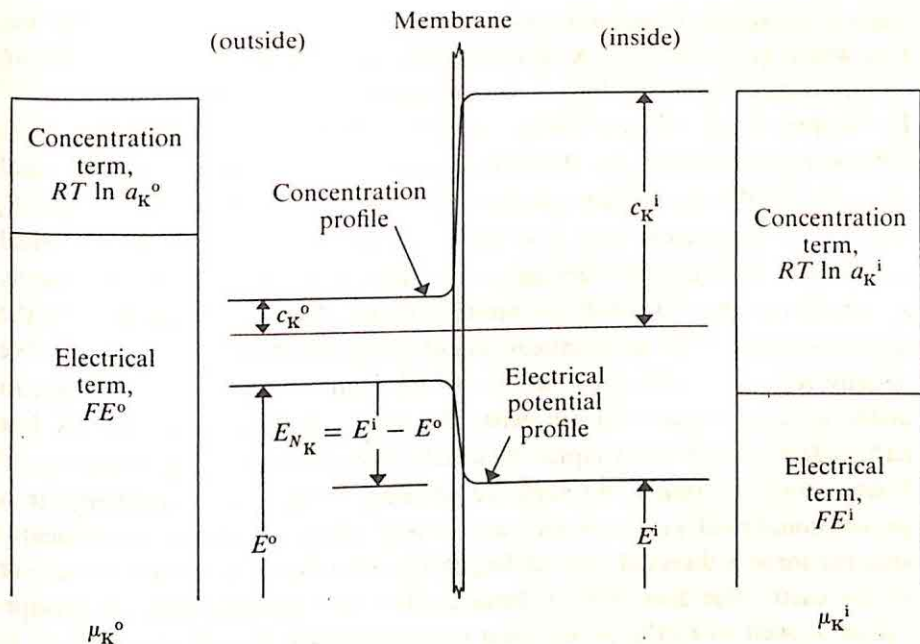


Figure 3.1

Equilibrium of K^+ across a membrane. When potassium is in equilibrium across some membrane, the side with the higher concentration must be at a lower electrical potential in order for its chemical potential to be unchanged ($\mu_K^o = \mu_K^i$) in crossing the membrane (μ_K^* is the same on the two sides of the membrane). The electrical potential difference across the membrane is then the Nernst potential for potassium, E_{N_K} . Note that E^o and E^i must be expressed relative to some arbitrary baseline for electrical potentials.

products of metabolism flow out of cells. The primary concern of this section will be the passive fluxes of ions toward lower chemical potentials. Although the mathematical expressions on the next few pages are rather formidable, the underlying approach is really quite straightforward. First, we indicate that the flux of some species is directly proportional to the driving force causing the movement. Next, the driving force is expressed in terms of the relevant components of the chemical potential. We then examine the consequences of having electroneutrality when there are simultaneous passive fluxes of more than one type of ion. This leads us to an expression describing the electrical potential difference across a membrane in terms of the properties of the ions penetrating it.

Before discussing the relation between fluxes and chemical potentials, we will briefly consider fluxes already mentioned or which may be familiar from

other contexts. In Chapter 1 we discussed Fick's first law of diffusion (Eq. 1.1), which says that the flux of (neutral) species j equals $-D_j \partial c_j / \partial x$, where we can consider that the force is the negative gradient of the concentration. In Chapter 8 we will use Darcy's law (Eq. 8.7) and Poiseuille's law (Eq. 8.9), both of which indicate that the volume flux of a solution is proportional to $-\partial P / \partial x$. We are all familiar with Ohm's law in the form ΔE equals IR , where I is the current and R is the resistance across which the electrical potential drop is ΔE . The current per unit area A is simply the flux of charge, J_c , which equals $-(1/\rho) \partial E / \partial x$ where ρ is the electrical resistivity and the negative gradient of the electrical potential represents the driving force. We usually replace $-\partial E / \partial x$ by $\Delta E / \Delta x$, which leads to $I/A = (1/\rho) \Delta E / \Delta x$. In order to make this conform with the usual expression of Ohm's law ($\Delta E = IR$), $\rho \Delta x / A$ must equal R , which indeed it does. The gravitational force, $-m_j g$, is simply the negative gradient of the potential energy in a gravitational field, i.e., $-\partial m_j g h / \partial h = -m_j g$, where the minus sign indicates that the force is directed toward decreasing altitudes, viz., toward the center of the earth. The gravitational force leads to the various forms of precipitation as well as to the percolation of water down through the soil.

We have considered examples of fluxes depending on each of the variable terms of the chemical potential ($\mu_j = \mu_j^* + RT \ln a_j + \bar{V}_j P + z_j F E + m_j g h$, Eq. 2.4). In particular, the activity term ($RT \ln a_j$) leads to Fick's first law, the pressure term ($\bar{V}_j P$) accounts for Darcy's law and Poiseuille's law, the electrical term ($z_j F E$) yields Ohm's law, and the gravitational term ($m_j g h$) is responsible for fluxes caused by gravity. In each case, flux is found by experiment to be directly proportional to the appropriate driving force. We can actually generalize such relationships since nearly all transport phenomena can be represented by the statement: flux equals an appropriate force divided by some resistance (flux = force/resistance). Force is the negative gradient of a suitable potential, which we often conveniently take as the change in potential over some distance. But we have already shown that the chemical potential is an elegant way of summarizing all the factors which can contribute to the motion of a substance. It should not be surprising, therefore, that *in general* the flux of species j is proportional to the negative gradient of its chemical potential, $-\partial \mu_j / \partial x$.

Flux and mobility. Let us consider a charged substance, species j , which can cross a particular membrane. At equilibrium, the chemical potential of species j , μ_j , does not change with time or position—we are dealing with communicating regions—and there is no net flux of this solute across the membrane. When μ_j changes with position but not with time, the situation is referred to as a *steady state*, a condition often used to approximate

problems of biological interest. Actually, it is a matter of judgment whether μ_j is constant enough in time to warrant a description of the system as being in a steady state. (Similarly, constancy of μ_j for appropriate time and distance intervals is necessary before indicating that a system is in equilibrium.) Here we will let μ_j change with both position and time over regions where species j is free to move; later we will restrict our development to the steady state case.

When μ_j depends on position, a net passive movement or flux of species j , J_j , will tend to occur toward the region where the chemical potential is lower. The negative gradient of the chemical potential of species j , $-\partial\mu_j/\partial x$, acts as the driving force for this J_j . (As in Ch. 1, the discussion will again apply to the one-dimensional case, thus we will let $\partial\mu_j/\partial y = \partial\mu_j/\partial z = 0$.) The greater $-\partial\mu_j/\partial x$ is, the larger will be the flux of species j in the x -direction. As a very useful approximation, we will assume that J_j is proportional to $-\partial\mu_j/\partial x$, where the minus sign means that a net positive flux occurs in the direction of decreasing chemical potential. The magnitude of a flux across some plane is also proportional to the local concentration of that species, c_j . That is, for a given driving force on species j , the amount of that substance which moves is proportional to how much of it is actually present—the more present, the greater the flux. Thus, for the one-dimensional case of crossing a plane perpendicular to the x -axis, J_j can be expressed as

$$J_j = u_j c_j \left(- \frac{\partial \mu_j}{\partial x} \right) \quad (3.6)$$

$$= \bar{v}_j c_j$$

where u_j is a coefficient called the *mobility* of species j . A mobility is generally the ratio of some velocity to the force which causes the motion—we will return to a consideration of u_j shortly.

As we have already indicated, the top line of Equation 3.6 is a representative example from the large class of expressions relating various flows to their causative forces. In this particular case, J_j is the rate of flow of moles of species j across unit area of a plane, and can be expressed in moles/cm²-sec. Such a molar flux of species j divided by its local concentration, c_j , gives the mean velocity, $\bar{v}_j$, with which this species moves across the plane—when c_j is in moles/cm³, J_j/c_j can have units of (moles/cm²-sec)/(moles/cm³) or cm/sec. Perhaps this important point can be better appreciated by considering it in the following way. Let us suppose that the average velocity of species j moving perpendicularly toward area A of the plane of interest is $\bar{v}_j$, and we will consider a volume element of cross-sectional area A which

extends back from the membrane for a distance $\bar{v}_j dt$. In a time dt , all molecules in the volume element $\bar{v}_j dt A$ will cross area A , which means that the number of moles of species j crossing in this interval is $(\bar{v}_j dt A) \times (c_j)$, where c_j is the number of moles/unit volume. Hence, the molar flux, J_j (which is simply the number of moles crossing unit area per unit time), would be $\bar{v}_j dt A c_j / (A dt)$, or $\bar{v}_j c_j$. In other words, the mean velocity of species j moving across the plane ($\bar{v}_j$) times the number of those molecules per unit volume which can move (c_j) equals the flux of that species (J_j), as is given by the bottom line of Equation 3.6. The upper line of Equation 3.6 indicates that this average velocity, J_j/c_j , equals the mobility of species j , u_j , times $-\partial\mu_j/\partial x$, the latter being the force on j that causes it to move. Thus, mobility is simply the proportionality factor between the mean velocity of motion ($\bar{v}_j = J_j/c_j$) and the causative force ($-\partial\mu_j/\partial x$). The greater the mobility of some species, the larger its velocity in response to a given force.

The particular form of the chemical potential of species j , μ_j , to be substituted into Equation 3.6 depends on the specific application that we have in mind. For charged particles moving across biological membranes, the appropriate μ_j is $\mu_j^* + RT \ln a_j + z_j FE$. (As we mentioned before, the $\bar{V}_j P$ term makes only a relatively small contribution to the $\Delta\mu_j$ of an ion, therefore it is not included.) For treating the one-dimensional case described by Equation 3.6, μ_j must be differentiated with respect to x , $\partial\mu_j/\partial x$, which leads to $RT \partial \ln a_j / \partial x + z_j F \partial E / \partial x$ when T is constant.* The quantity $\partial \ln a_j / \partial x$ equals $(1/a_j) \partial a_j / \partial x$, which is $(1/\gamma_j c_j) \partial \gamma_j c_j / \partial x$ ($a_j = \gamma_j c_j$, Eq. 2.5). Using the above form of μ_j appropriate for charged solutes and this expansion of the derivative, $\partial \ln a_j / \partial x$, the net flux of species j appearing in Equation 3.6 can be written

$$J_j = -\frac{u_j RT}{\gamma_j} \frac{\partial \gamma_j c_j}{\partial x} - u_j c_j z_j F \frac{\partial E}{\partial x} \quad (3.7)$$

Equation 3.7 becomes simply $-u_j RT (\partial c_j / \partial x)$, i.e., it is proportional to the j either across a membrane or in a solution in terms of two components of the driving force: the gradients in activity and electrical potential.

Before proceeding, we should examine a little more closely the first term on the right side of Equation 3.7. When γ_j varies across the membrane, $\partial \gamma_j / \partial x$ can be considered to represent a driving force on species j . In keeping with common practice, we will ignore this possible force, i.e., we will assume that γ_j can be treated as a constant—in any case $\partial \gamma_j / \partial x$ would be extremely

* Actually, we must assume isothermal conditions in order for $-\partial\mu_j/\partial x$ to represent the force precisely, i.e., T is a constant here, hence $\partial (RT \ln a_j) / \partial x = RT \partial \ln a_j / \partial x$.

difficult to measure. For constant γ_j , the first term on the right side of Equation 3.7 becomes simply $-u_j RT(\partial c_j/\partial x)$, i.e., it is proportional to the concentration gradient. In the absence of electrical potential gradients ($\partial E/\partial x = 0$), and for neutral solutes ($z_j = 0$), Equation 3.7 indicates that J_j equals $-u_j RT(\partial c_j/\partial x)$. But this is just the type of flux described by Fick's first law ($J_j = -D_j \partial c_j/\partial x$, Eq. 1.1), with $u_j RT$ taking the place of the diffusion coefficient, D_j . In other words, we can replace $u_j RT$ in Equation 3.7 by D_j . Since D_j equals $u_j RT$, diffusion coefficients must depend on temperature. Moreover, u_j is generally inversely proportional to the viscosity, which decreases as T increases, thus the dependence of D_j on T can be pronounced. Consequently, the temperature should be specified when the value of a diffusion coefficient is given.

Let us now return to a consideration of fluxes. In the absence of electrical effects and for constant γ_j , the net flux of species j given by Equation 3.7 equals $-D_j \partial c_j/\partial x$ when $u_j RT$ is replaced by D_j . Thus, Fick's first law ($J_j = -D_j \partial c_j/\partial x$, Eq. 1.1) is a special case of our general flux relation (Eq. 3.6), where we ignored first the pressure and gravitational effects in order to obtain Equation 3.7 and then the electrical effects. But this eventual reduction of Equation 3.6 to Fick's first law is as we should expect, since in fact the only driving force considered when we presented Fick's first law (Ch. 1) was the concentration gradient. Such agreement between our present thermodynamic approach and the *seemingly* more empirical Fick's first law is quite important. It serves to justify the logarithmic term for activity in the chemical potential ($\mu_j = \mu_j^* + RT \ln a_j + \bar{V}_j P + z_j FE + m_j gh$, Eq. 2.4). In other words, if the activity of species j appeared in Equation 2.4 in a form other than $\ln a_j$, Equation 3.7 would not reduce to Fick's first law under the appropriate conditions. Since Fick's first law has been amply demonstrated experimentally, such a disagreement between theory and practice would necessitate some modification in the expression used to define chemical potential.

As indicated in Chapter 2, the terms in the chemical potential can be justified or "derived" by different methods. The forms of some terms in μ_j can be readily appreciated since they follow from familiar definitions of work, e.g., the gravitational term (p. 62) and the electrical term (p. 92). The above comparison with Fick's first law indicates that the $\ln a_j$ form is the appropriate way of handling the activity term. Another derivation of the $RT \ln a_j$ term will be found in Appendix VII, together with a discussion of the pressure term for both liquids and gases. Some of these derivations incorporate conclusions from empirical observations. Moreover, the fact that the chemical potential can be expressed as a series of terms which can be

added together agrees with experiment. Thus, a thermodynamic expression for the chemical potential such as Equation 2.4 can (1) summarize the results of previous observations (2) stand the test of experiments, and (3) lead to new and useful predictions.

In contrast to the case for a neutral solute, the flux of an ion also depends on an electrical driving force, represented in Equation 3.7 by $-\partial E/\partial x$. A charged solute spontaneously tends to move in an electrical potential gradient—a cation moving in the direction of lower or decreasing electrical potential. Of course, the concentration gradient also affects charged particles. If a certain type of ion were present in some region but absent in an adjacent one, the ions would tend to diffuse into the latter region. As such charged particles diffuse toward regions of lower concentration, an electrical potential difference is created. This electrical potential difference is referred to as a “diffusion potential,” which we will discuss in detail below. The interrelationship between concentration and electrical effects is extremely important in biology. Most membrane potentials can be treated as diffusion potentials resulting from different rates of movement of the various ions across a membrane.

Diffusion potential in a solution. We will now use Equation 3.7 to derive the electrical potential difference created by ions diffusing down a concentration gradient in a solution containing one type of cation and its accompanying anion. This case is relatively simple compared with the more biological one to follow, thus it may more clearly illustrate the relationship between concentration gradients and the accompanying electrical potential differences. We will assume that the cations and anions are initially placed at one side of the solution. In time, they will *diffuse* across the solution toward regions of lower concentration. In general, one ionic species will have a higher mobility, u_j , than the other. The more mobile ions will tend to diffuse faster than their oppositely charged partners, resulting in a microscopic charge separation. This slight charge separation sets up an electrical potential gradient leading to a *diffusion potential*. Using certain simplifying assumptions, we will calculate the magnitude of the electrical potential difference so created.

For convenience of analysis, let us consider a solution containing only a monovalent cation (+) and its monovalent anion (−). We will assume that their activity coefficients are constant. As the previous calculations on electrical effects indicated, solutions are essentially neutral in regions which are large compared with atomic dimensions. Thus, c_+ equals c_- , and the concentration of either species can be designated c . Furthermore, no charge imbalance will develop in time, which means that the flux of the cation across some plane in the solution, J_+ , equals that of the anion across the same plane,

J_- . (A very small charge imbalance does develop, which sets up the electrical potential gradient, but this uncompensated flux is transitory and in any case negligible compared with J_+ or J_- .) Both fluxes, J_+ and J_- , can be expressed by Equation 3.7 and then equated to each other, which gives

$$-u_+ RT \frac{\partial c}{\partial x} - u_+ c F \frac{\partial E}{\partial x} = -u_- RT \frac{\partial c}{\partial x} + u_- c F \frac{\partial E}{\partial x} \quad (3.8)$$

where the plus sign on the right side occurs because the monovalent anion carries a negative charge ($z_- = -1$). Rearrangement of Equation 3.8 yields the following expression for the electrical potential gradient:

$$\frac{\partial E}{\partial x} = \frac{u_- - u_+}{u_+ + u_-} \frac{RT}{Fc} \frac{\partial c}{\partial x} \quad (3.9)$$

Equation 3.9 indicates that a nonzero $\partial E/\partial x$ occurs when the mobility of the cation differs from that of the anion and there is a concentration gradient. If u_- is greater than u_+ , the anions move (diffuse) faster than the cations toward regions of lower concentration. As some individual anion moves ahead of its "partner" cation, an electric field is set up in such a direction as to speed up the cation and slow down the anion until they both move at the same speed, thus preserving electrical neutrality.

In order to obtain the difference in electrical potential produced by diffusion between planes of differing concentration, we must integrate Equation 3.9. At this point we will restrict our consideration to the *steady state* case where neither E nor c changes with time. In going along the x axis from region I to region II, the change in the electrical potential term in Equation 3.9 is $\int_I^{II} (\partial E/\partial x) dx$, which becomes $\int_I^{II} dE$ for the steady state condition* and so equals $E^{II} - E^I$. The integral of the concentration term is $\int_I^{II} (1/c)(\partial c/\partial x) dx$, which becomes $\int_I^{II} dc/c$, or $\ln(c^{II}/c^I)$. Using these two relations, integration of Equation 3.9 leads to

$$\begin{aligned} E^{II} - E^I &= \frac{u_- - u_+}{u_+ + u_-} \frac{RT}{F} \ln \frac{c^{II}}{c^I} \\ &= 59.2 \frac{u_- - u_+}{u_+ + u_-} \log \frac{c^{II}}{c^I} \text{ mv at } 25^\circ \end{aligned} \quad (3.10)$$

* As we discuss in Appendix VI (Eq. VI.5), a total derivative like dE equals $(\partial E/\partial x)dx + (\partial E/\partial t)dt$, while dc is $(\partial c/\partial x)dx + (\partial c/\partial t)dt$. For a steady state condition, both $\partial E/\partial t$ and $\partial c/\partial t$ are zero. Consequently, dE then equals $(\partial E/\partial x)dx$ and dc equals $(\partial c/\partial x)dx$.

where in the second line $\ln$ has been replaced by $2.303 \log$, and the value 59.2 mv at 25° has been substituted for $2.303 RT/F$ (App. III). In the general case, the anions have mobilities different from the cations. As the ions diffuse to regions of lower chemical potential in the solution, an electrical potential difference—given by Equation 3.10, and called a diffusion potential—is set up across the section where the concentration changes from c^I to c^{II} .

An example of a diffusion potential describable by Equation 3.10 occurs at the open end of the special micropipettes used for measuring electrical potential differences across membranes. (The “pulling,” filling, and using of micropipettes—commonly referred to as microelectrodes or microsalt-bridges—are described in App. IX.) The fine tip of the micropipette is open and provides an electrically conducting pathway into the cell or tissue. Ions diffusing from this fine tip give rise to a diffusion potential between the interior of the micropipette and the aqueous compartment into which the tip is inserted. To estimate the magnitude of this potential, we will assume that there is a concentration ratio of 30 across the micropipette tip, and then calculate the diffusion potentials for typical electrolytes, NaCl and KCl. The chloride mobility, u_{Cl} , is about 1.52 times u_{Na} and so $(u_- - u_+)/ (u_+ + u_-)$ is $(0.52)/(2.52)$, or 0.21 for NaCl (see Briggs *et al.*). When the concentration ratio is 30, the diffusion potential calculated by Equation 3.10 is $(59.2 \text{ mv})(0.21) \log (1/30)$, or -18 mv . For KCl as the electrolyte, u_{Cl} is $1.04u_{\text{K}}$, and thus $(u_- - u_+)/ (u_+ + u_-)$ is $(0.04)/(2.04)$, or 0.02. This leads to a diffusion potential of $(59.2)(0.02) \log (1/30)$ or only -2 mv in going from the interior of the micropipette, where the concentration of KCl is, say, 3 M, to a region where it is 0.1 M. Thus, from the point of view of minimizing the diffusion potential across the fine tip, KCl is a much more suitable electrolyte for micropipettes than is NaCl. In fact, KCl is employed in nearly all micropipettes used for measuring membrane potentials (generally c_{K} inside cells is also higher than c_{Na} , and it does not vary as much from cell to cell as does c_{Na}). In any case, the closer u_- is to u_+ , the smaller will be the diffusion potential predicted by Equation 3.10 for a given concentration ratio.

Membrane fluxes. As is the case for diffusion potentials in open solution, membrane potentials also depend on the different mobilities of the various ions and on their concentration gradients. In this case, however, the “solution” in which the diffusion takes place toward regions of lower chemical potential is actually the membrane itself. In this regard, we noted in Chapter 1 that a membrane is often the main barrier, or rate-limiting step for the diffusion of molecules into and out of cells or organelles. We would therefore expect it to be the phase across which the diffusion potential is expressed.

Under biological conditions a number of different types of ions are present, hence the situation is more complex than for the single cation-anion pair analyzed above. Furthermore, the quantities of interest such as $\partial E/\partial x$ and γ_j are those within the membrane, hence they are not accessible to experimental measurement. We will consider the ways of dealing with these difficulties in this section.

In order to calculate membrane diffusion potentials, we must make certain assumptions before an integration of fluxes or gradients can be made. As a start, we will assume that the electrical potential (E) varies linearly with distance across the membrane. This means that $\partial E/\partial x$ is a constant equal to $E_M/\Delta x$, where E_M is the electrical potential difference across the membrane—i.e., $E^i - E^o = \Delta E = E_M$, as we indicated on p. 99—and Δx is the membrane thickness. This assumption of a constant electric field across the membrane—the electric field equals $-\partial E/\partial x$ in our one-dimensional case—was originally suggested by D. E. Goldman in 1943 (see Goldman, or Hodgkin and Katz). It appreciably simplifies the integration conditions leading to an expression describing the electrical potential difference across biological membranes. As another very useful approximation, we will assume that the activity coefficient of species j , γ_j , is constant across the membrane (a less severe restriction than setting it equal to one). As we noted in Chapter 1, a partition coefficient is needed to describe concentrations within a membrane since the solvent properties of a membrane are different from those of the aqueous solutions on either side of it, where the concentrations are actually determined. Thus, c_j in Equation 3.7 should be replaced by $K_j c_j$, where K_j is the partition coefficient for species j . Incorporating these various simplifications and conditions, and after transferring the electrical term to the left side, we can rewrite Equation 3.7 as follows:

$$u_j K_j c_j z_j F \frac{E_M}{\Delta x} + J_j = -u_j R T K_j \frac{\partial c_j}{\partial x} \quad (3.11)$$

In order to cast Equation 3.11 into a form that can be integrated, it is advantageous to put all terms containing the concentration on the same side of the equation. Furthermore, since the two most convenient variables are c_j and x , their differentials should appear on opposite sides of the equation. Being guided by these two objectives, and after first multiplying each term by $dx/(u_j R T)$, we can rearrange Equation 3.11 to give the following expression:

$$\frac{z_j F E_M}{R T \Delta x} dx = - \frac{K_j dc_j}{K_j c_j + \frac{J_j \Delta x}{u_j z_j F E_M}} \quad (3.12)$$

where $(\partial c_j / \partial x) dx$ has been replaced by dc_j in anticipation of the restriction to steady-state conditions (cf. footnote to p. 107).

When the term $J_j \Delta x / (u_j z_j F E_M)$ is constant, Equation 3.12 can be readily integrated from one side of the membrane to the other. The factors Δx , z_j , F , and E_M are all fixed, and only J_j and u_j need be further considered here. When the flux of species j does not change with time or position, then J_j across any plane parallel to and within the membrane is the same, and species j is not accumulating or being depleted in any of the regions of interest. Consequently, $\partial c_j / \partial t$ is zero, while of course $\partial c_j / \partial x$ is nonzero, which is the steady state condition (cf. footnote to p. 107). Our restriction to a steady state therefore means that J_j is constant and $(\partial c_j / \partial x) dx$ equals dc_j , a relation which we have already incorporated into Equation 3.12. For convenience, the mobility (u_j) of each species is also assumed to be constant within the membrane. With these restrictions, the quantity $J_j \Delta x / (u_j z_j F E_M)$ becomes a constant, and we can integrate Equation 3.12 from one side of the membrane (the outside, o) to the other (the inside, i). By Appendix VI, $\int dx / (x + b)$ equals $\ln (x + b)$. Hence,

$$- \int_{K_j c_j^o}^{K_j c_j^i} K_j dc_j / (K_j c_j + b)$$

is

$$- \ln (K_j c_j + b) \Big|_{K_j c_j^o}^{K_j c_j^i}$$

or $-\ln (K_j c_j^i + b) / (K_j c_j^o + b)$, which equals $\ln (K_j c_j^o + b) / (K_j c_j^i + b)$.

Since $\int_{x^o}^{x^i} dx$ equals Δx , integration of Equation 3.12 gives

$$\frac{z_j F E_M}{RT} = \ln \frac{\left(K_j c_j^o + \frac{J_j \Delta x}{u_j z_j F E_M} \right)}{\left(K_j c_j^i + \frac{J_j \Delta x}{u_j z_j F E_M} \right)} \quad (3.13)$$

After taking exponentials of both sides of Equation 3.13 in order to put it into a more convenient form, and then multiplying by $K_j c_j^i + J_j \Delta x / (u_j z_j F E_M)$, Equation 3.13 becomes

$$K_j c_j^i e^{z_j F E_M / RT} + \frac{J_j \Delta x}{u_j z_j F E_M} e^{z_j F E_M / RT} = K_j c_j^o + \frac{J_j \Delta x}{u_j z_j F E_M} \quad (3.14)$$

A quantity of considerable interest in Equation 3.14 is J_j , the net flux of species j . This equation can be readily solved for J_j , giving

$$J_j = J_j^{\text{in}} - J_j^{\text{out}} = \left(\frac{K_j u_j z_j F E_M}{\Delta x} \right) \left(\frac{1}{e^{z_j F E_M / RT} - 1} \right) (c_j^o - c_j^i e^{z_j F E_M / RT}) \quad (3.15)$$

where J_j^{in} is the influx of species j , J_j^{out} is its efflux, and their difference is the net flux. (The net flux J_j can represent either a net influx or a net efflux, depending on which of the unidirectional components, J_j^{in} or J_j^{out} , is larger.) Although the mathematical manipulations necessary to go from Equation 3.11 to Equation 3.15 are lengthy and cumbersome, the resulting expression is of extreme importance for understanding both membrane potentials and the passive fluxes of ions. Moreover, throughout this text all the steps involved in a particular derivation have been shown in order to avoid statements such as "it can *easily* be shown that such and such follows from so and so"—expressions which can be most frustrating and often are untrue. Equation 3.15 will be used to derive the Goldman equation—describing the diffusion potential across membranes—and will be invoked later in the derivation of the Ussing-Teorell equation, a relation obeyed by certain passive fluxes.

Equation 3.15 shows how the passive flux of some charged species j depends on its internal and external concentrations and on the electrical potential difference across the membrane. For most cell membranes, E_M is negative, i.e., the inside of the cell is at a lower electrical potential than is the outside. For a cation (z_j a positive integer) and a negative E_M , the terms in the first two parentheses on the right side of Equation 3.15 are both negative, hence their product is positive. On the other hand, for an anion (z_j a negative integer) and a negative E_M , both parentheses are positive, and their product is still positive. (For $E_M > 0$, the product of the first two parentheses is also positive for both anions and cations.) Thus, the sign of J_j depends on the value of c_j^o relative to that of $c_j^i e^{z_j F E_M / RT}$. When c_j^o is greater than $c_j^i e^{z_j F E_M / RT}$, the expression in the last parenthesis of Equation 3.15 is positive, and a net inward flux of species j occurs ($J_j > 0$). Such a condition may be contrasted with Equation 1.8 [$J_j = P_j(c_j^o - c_j^i)$], where the net flux in the absence of electrical effects was inward when c_j^o was simply larger than c_j^i , as would adequately describe the situation for neutral solutes. However, knowledge of the concentration difference alone is not sufficient to predict the magnitude or even the direction of the flux of ions; we must also consider the electrical potential difference between the two regions.

Membrane diffusion potential—Goldman equation. Passive fluxes of ions, which can be described by Equation 3.15 and are caused by gradients in the chemical potentials of the various species, lead to an electrical potential difference (diffusion potential) across a membrane. We can determine the magnitude of this electrical potential difference by considering the contributions from all ionic fluxes across the membrane and the condition of electroneutrality. Certain assumptions are needed, however, to keep the

equations manageable. Under usual biological situations not all anions and cations can easily move through the membranes. Many divalent cations do not readily enter or leave plant cells passively, which means that their mobility in the membranes is small. Such ions usually do not make a large enough contribution to the fluxes into or out of plant cells to influence markedly the diffusion potentials across the membranes. Thus, we will omit them in the present analysis, which is nevertheless rather complicated.

For many plant cells, the total ionic flux consists mainly of movements of K^+ , Na^+ , and Cl^- (see Dainty 1962, Gutknecht and Dainty, or Hope). These three ions generally have fairly high concentrations in and near plant cells, and we would therefore expect them to make substantial contributions to the total ionic flux. More specifically, a flux of species j depends on the product of its concentration and its mobility ($J_j = -u_j c_j \partial \mu_j / \partial x$, Eq. 3.6), thus, ions having relatively high local concentrations or moving in the membrane fairly easily (high u_j) will tend to be the major contributors to the total ionic flux. In some cases there may be a sizable flux of H^+ or OH^- (which can have high u_j s) as well as other ions, the restriction here to three ions being partially in the interest of algebraic simplicity. However, the real justification for considering only K^+ , Na^+ , and Cl^- is that the diffusion potentials calculated by using the passive fluxes of these three ions across biological membranes are often in good agreement with the measured electrical potential differences.

Our previous electrical calculations indicated that solutions are essentially neutral, a condition which we will again invoke here. In other words, because of the large effects resulting from small amounts of uncanceled charge, the net charge actually needed to cause the electrical potential difference across a membrane (E_M) is negligible compared with the existing concentrations of ions. Furthermore, the steady-state fluxes of the ions across the membrane will not change this condition of electrical neutrality, which means that no net charge is transported by the algebraic sum of the various charge movements across the membrane, i.e., $\sum_j z_j J_j = 0$. When the bulk of the ionic flux consists of K^+ , Na^+ , and Cl^- movements, this important condition of electroneutrality can be described by equating the cationic fluxes ($J_K + J_{Na}$) to the anionic one (J_{Cl}), which leads to the following relation (as mentioned above, the various ions are indicated by using only their element symbols as subscripts):

$$J_K + J_{Na} - J_{Cl} = 0$$

(3.16)

Equation 3.16 describes the net ionic fluxes which lead to the electrical potential difference across the membrane. After substituting the expressions

for the various J_j s into Equation 3.16, we will solve the resulting equation for the diffusion potential across the membrane, E_M .

To obtain a useful expression for E_M in terms of measurable parameters, it is convenient to introduce a permeability coefficient for species j , P_j . In Chapter 1, such a permeability coefficient was defined as $D_j K_j / \Delta x$, where D_j is the diffusion coefficient of species j , K_j is its partition coefficient, and Δx is the membrane thickness (Eq. 1.9). Upon comparing Equation 3.7 with Equation 1.1 ($J_j = -D_j \partial c_j / \partial x$), we can see that $u_j RT$ takes the place of the diffusion coefficient of species j , D_j , as pointed out on p. 105. The quantity $K_j u_j RT / \Delta x$ can thus be equated to $K_j D_j / \Delta x$, which is the permeability coefficient (P_j) of species j for some particular membrane. In this way, the unknown mobility of species j in a given membrane, the unknown thickness of the membrane, and the partition coefficient for the solute can be replaced by one parameter, which describes the permeability properties of that solute crossing the particular membrane under consideration.

With all the preliminaries out of the way, let us now determine the expression for the diffusion potential across a membrane for the case where most of the ionic flux is due to K^+ , Na^+ , and Cl^- movements. Using the permeability coefficients of the three ions and substituting in the net flux of each species as defined by Equation 3.15, Equation 3.16 becomes

$$P_K \left(\frac{1}{e^{FE_M/RT} - 1} \right) (c_K^o - c_K^i e^{FE_M/RT}) + P_{Na} \left(\frac{1}{e^{FE_M/RT} - 1} \right) \times (c_{Na}^o - c_{Na}^i e^{FE_M/RT}) + P_{Cl} \left(\frac{1}{e^{-FE_M/RT} - 1} \right) (c_{Cl}^o - c_{Cl}^i e^{-FE_M/RT}) = 0 \quad (3.17)$$

where z_K and z_{Na} have been replaced by 1, z_{Cl} by -1 , and FE_M/RT has been canceled from each of the terms for the three net fluxes. To simplify this rather unwieldy expression, the quantity $1/(e^{FE_M/RT} - 1)$ can be canceled from each of the three terms represented in Equation 3.17— $1/(e^{-FE_M/RT} - 1)$ appearing in the last term is the same as $-e^{FE_M/RT}/(e^{FE_M/RT} - 1)$. Equation 3.17 then assumes a much more manageable form:

$$P_K c_K^o - P_K c_K^i e^{FE_M/RT} + P_{Na} c_{Na}^o - P_{Na} c_{Na}^i e^{FE_M/RT} - P_{Cl} c_{Cl}^o e^{FE_M/RT} + P_{Cl} c_{Cl}^i = 0 \quad (3.18)$$

After solving Equation 3.18 for $e^{FE_M/RT}$ and taking logarithms, we obtain the following expression for the electrical potential difference across the membrane:

$$E_M = \frac{RT}{F} \ln \frac{(P_K c_K^o + P_{Na} c_{Na}^o + P_{Cl} c_{Cl}^i)}{(P_K c_K^i + P_{Na} c_{Na}^i + P_{Cl} c_{Cl}^o)} \quad (3.19)$$

Equation 3.19 is generally known as the Goldman, or constant field equation. As we mentioned on p. 109, the electric field equals $-\partial E/\partial x$, which Goldman in 1943 set equal to a constant (here $-E_M/\Delta x$) in order to facilitate the integration across a membrane. It turns out that the assumption of a constant electric field in the membrane is actually not essential for obtaining Equation 3.19; we could invoke Gauss's law and perform a more difficult integration. (See Goldman for a consideration of the constant field situation in a general case.) In 1949, Hodgkin and Katz applied the general equation derived by Goldman to the specific case of K^+ , Na^+ , and Cl^- diffusing across a membrane (see Hodgkin and Katz), and Equation 3.19 is often referred to as the Goldman-Hodgkin-Katz equation.

Equation 3.19 gives the diffusion potential existing across a membrane. We have derived it by assuming independent passive movements of K^+ , Na^+ , and Cl^- across a membrane in which $\partial E/\partial x$, γ_j , J_j , and u_j are all constant. We used the negative gradient of its chemical potential as the driving force for the net flux of each ion. Thus, Equation 3.19 gives the electrical potential difference arising from the different tendencies of K^+ , Na^+ , and Cl^- to *diffuse* across a membrane to regions of lower chemical potential. When other ions cross some particular membrane in appreciable amounts they will also make a contribution to its membrane potential. However, the inclusion of divalent and trivalent ions in the derivation of an expression for E_M complicates the algebra considerably (e.g., if Ca^{++} is also considered, Eq. 3.18 has fourteen terms on the left side instead of six, and the equation becomes a quadratic in powers of $e^{FE_M/RT}$). The fluxes of such ions may be rather small, in which case Equation 3.19 may be adequate for describing the membrane potential.

Application of the Goldman equation. In certain cases, the terms appearing in Equation 3.19, viz., the permeabilities and the internal and external concentrations of K^+ , Na^+ , and Cl^- , have all been measured. The validity of the Goldman equation can then be checked. In other words, we can compare the predicted diffusion potential with the actual electrical potential difference experimentally measured across the membrane.

As a specific example, we will use the Goldman equation to evaluate the membrane potential across the plasmalemma of *Nitella translucens*. The concentrations of K^+ , Na^+ , and Cl^- in the external bathing solution and in the cytoplasm of *Nitella translucens* are given in Table 3.1. The ratio of the permeability of Na^+ to that of K^+ , P_{Na}/P_K , is about 0.18 for *Nitella translucens* (see MacRobbie 1962). The plasmalemma of *Nitella* is much less permeable to Cl^- than it is to K^+ , probably only 0.1% to 1% as much. Thus, for purposes of calculation, we will let P_{Cl}/P_K be 0.003. In terms of perme-

abilities relative to that for potassium, P_K for *Nitella* is then 1.00, P_{Na} is 0.18, and P_{Cl} is 0.003. Using these relative permeability coefficients and the concentrations given in Table 3.1, the argument of the logarithm appearing in Equation 3.19, $(P_K c_K^o + P_{Na} c_{Na}^o + P_{Cl} c_{Cl}^i) / (P_K c_K^i + P_{Na} c_{Na}^i + P_{Cl} c_{Cl}^o)$, has the numerical value of $[(1.0)(0.1 \text{ mM}) + (0.18)(1.0 \text{ mM}) + (0.003)(65 \text{ mM})]$ divided by $[(1.0)(119 \text{ mM}) + (0.18)(14 \text{ mM}) + (0.003)(1.3 \text{ mM})]$, which is $(0.475)/(121.5)$, or 0.00391. Since $\ln$ is 2.303 $\log$ and $2.303 RT/F$ equals 58.2 mv at 20° (App. III), the Goldman equation predicts a membrane potential, E_M , of $(58.2 \text{ mv}) \log (0.00391)$, or -140 mv . Thus, we expect the cytoplasm to be electrically negative with respect to the external bathing solution, as is indeed the case. In fact, the measured value of the electrical potential difference across the plasmalemma of *Nitella translucens* is -138 mv at 20° (Table 3.1). This excellent agreement between the observed electrical potential difference and that calculated from the Goldman equation supports the contention that the membrane potential is in fact a diffusion potential. This point can be fairly easily checked by varying the external concentration of K^+ , Na^+ , and/or Cl^- and then seeing whether the membrane potential changes in accordance with Equation 3.19. (App. IX discusses the actual measurement of E_M under various experimental conditions, as well as interpretations of E_M using the Goldman equation.)

The different ionic concentrations on the two sides of a membrane help set up the passive ionic fluxes creating the diffusion potential, but the actual magnitude of the contribution of a particular ionic species to E_M also depends on the ease with which that type of ion can cross the membrane. Based on the relative permeabilities and concentrations, the major contribution to the electrical potential difference across the plasmalemma of *Nitella* comes from the K flux, with Na and Cl playing secondary roles. If the Cl terms are omitted from Equation 3.19 (i.e., if P_{Cl} is set equal to zero), the calculated membrane potential is -153 mv , compared with -140 mv when Cl is included. This rather small difference between the two potentials is a reflection of the relatively low permeability coefficient for chloride crossing the plasmalemma of *Nitella*, hence the Cl flux has less effect on E_M than does the K flux.

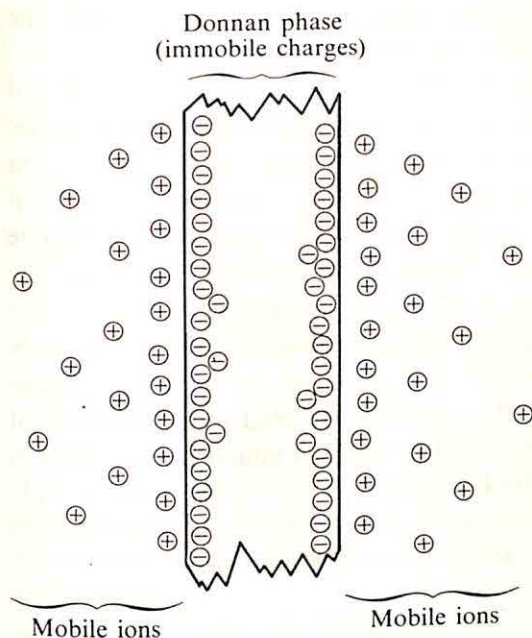
Changes in the amount of Ca^{++} or H^+ in the external medium cause some deviations from the predictions of Equation 3.19 for the electrical potential difference across the plasmalemma of *Nitella*. Thus, the diffusion potential is influenced by the properties of the particular membrane being considered, and ions other than K^+ , Na^+ , and Cl^- may have to be included in specific cases. To allow for the influence of the flux of H^+ on E_M , for example, we could include $P_H c_H^o$ in the numerator of the logarithm in the

Goldman equation (Eq. 3.19) and $P_H c_H^i$ in the denominator (note that a movement of H^+ in one direction has the same effect on E_M as a movement of OH^- in the other). Nevertheless, restriction to the three ions indicated has proved to be adequate for treating the diffusion potential across many membranes, and the Goldman, or constant field expression in the form of Equation 3.19 has found widespread application.

Donnan potential. Another type of electrical potential difference encountered in biological systems is associated with immobile or fixed charges in some region adjacent to an aqueous phase containing small mobile ions, and is referred to as a *Donnan potential*. When a plant cell is placed in a KCl solution, for example, a Donnan potential arises between the interior of the cell wall and the bulk of the bathing fluid. The electrical potential difference arising from electrostatic interactions at such a solid-liquid interface can be regarded as a special type of diffusion potential, as we will show shortly.

Pectin and other macromolecules in the cell wall have a large number of immobile carboxyl groups ($-COOH$) from which hydrogen ions dissociate. This gives the cell wall a net negative charge, as indicated in Chapter 1. Cations such as Ca^{++} are electrostatically attracted to the negatively charged cell wall, and the overall effect is an exchange of H^+ for Ca^{++} and other cations.* The region containing the immobile charges—such as carboxyl groups in the case of the cell wall—is generally referred to as the *Donnan phase* (see Fig. 3.2). At equilibrium, a distribution of oppositely charged ions electrostatically attracted to these immobile charges occurs between the Donnan phase and the adjacent aqueous one (Fig. 3.2). This sets up an electrical potential gradient and a Donnan potential is thus created between the center of the Donnan phase and the bulk of the solution next to it. The sign of the electrical potential in the Donnan phase relative to the surrounding electrolyte solution is the same as the sign of the charge of the immobile ions. For example, because of the presence of dissociated carboxyl groups, the electrical potential in the cell wall is negative with respect to an external solution. Membranes also generally act as charged Donnan phases (see Lakshminarayanaiah). In addition, Donnan phases occur in the cytoplasm, where the immobile charges are due to proteins and other large polymers (RNA, DNA) that have many carboxyl and phosphate

* Such an attraction of positively charged species to the cell wall can increase the local concentration of solutes to about 0.6 M, and consequently a greater osmotic pressure generally exists in the cell wall than in the surrounding aqueous solutions, as indicated on p. 85 (see Dainty and Hope as well as Spanner).

**Figure 3.2**

Spatial distribution of positively charged mobile ions (⊕) occurring on either side of a Donnan phase in which are embedded immobile negative charges (⊖).

groups from which protons can dissociate, leaving the macromolecules with a net negative charge. Such polymers are fixed in the sense that they are unable to diffuse across either the plasmalemma or the tonoplast.

When the immobile or fixed ions are in some region, mobile ions of opposite sign can occur in adjacent layers on either side (Fig. 3.2). For example, layers containing cations are often formed in the aqueous solutions on each side of biological membranes, which generally act as Donnan phases having a net negative charge at usual pH's.* At equilibrium no net movement of the ions occurs, so the chemical potentials of each of the mobile ions (e.g., K^+ , Na^+ , Cl^- , Ca^{++}) have the same values up close to the Donnan phase as they do in the surrounding aqueous phase. The electrical potential difference (Donnan potential) can then be calculated by assuming constancy of the chemical potential. But this is exactly the same principle that we used in deriving the Nernst potential, $E_{N_j} = E^{\text{II}} - E^{\text{I}} = (RT/z_j F) \ln (a_j^{\text{I}}/a_j^{\text{II}})$, Equation 3.5, between two aqueous compartments. In fact, since the argument again hinges on the constancy of the chemical potential, the equilibrium distribution of any ion from the Donnan phase

* The electrical potential drops or Donnan potentials on either side of a membrane (cf. Fig. 3.2) are in opposite directions and therefore are *assumed* to cancel out when a diffusion potential across the membrane is calculated.

amount of charge transported in time t across the surface of the sphere (area = $4\pi r^2$) is $J_{a.t.Cl}^{in} 4\pi r^2 t$, where r is the radius. This active uptake of Cl^- increases the internal concentration of negative charge by the amount moved in divided by the cellular volume, or $J_{a.t.Cl}^{in} 4\pi r^2 t / (4\pi r^3 / 3)$, which is $3J_{a.t.Cl}^{in} t / r$. Let us suppose that $J_{a.t.Cl}^{in}$ is 1.0 picomole/cm²-sec (a picomole = 10^{-12} mole, App. IV) and that the cell has a radius of 30 μ m. In 1 sec the concentration of Cl^- actively transported in would then be $(3)(10^{-12} \text{ mole/cm}^2\text{-sec})(1 \text{ sec}) / (30 \times 10^{-4} \text{ cm})$, or $1.0 \times 10^{-9} \text{ mole/cm}^3$. Assuming a membrane capacitance of 1 μ f/cm², we calculated on p. 95 that such a cell has 1.0×10^{-9} mole of uncompensated negative charge/cm³ when the interior is 100 mv negative with respect to the external solution. If no change were to take place in the other ionic fluxes, the electrogenic uptake of Cl^- into this cell would cause its interior to become more negative at the rate of 100 mv/sec. The charging of the membrane capacitance is indeed a rapid process.

The initiation of an electrogenic process causes an adjustment of the passive fluxes across the membrane. In particular, the net charge actively brought in is electrically compensated for very soon by appropriate passive movements of other ions into or out of the cell. The actual electrical potential difference across the membrane then results from the diffusion potential caused by these new passive fluxes plus a contribution from the electrogenic process involving the active transport of some charged species. We can represent the electrical potential drop generated by the active transport of species j , $E_{a.t.j}$, as follows:

$$E_{a.t.j} = z_j F J_{a.t.j} R^{\text{memb}} \quad (3.20)$$

where z_j is the charge number of species j , F is the Faraday, $J_{a.t.j}$ is the net flux of species j caused by active transport, and R^{memb} is the membrane resistance. $F J_{a.t.j}$ in Equation 3.20—which is really a form of Ohm's law—has units of (coulombs/mole) $\times$ (moles/cm²-sec) or coulombs/cm²-sec, which is amperes/cm². Hence, R^{memb} must be expressed in ohms-cm², so that $z_j F J_{a.t.j} R^{\text{memb}}$ in Equation 3.20 can have units of ampere-ohms, i.e., volts, the proper unit for electrical potentials. For most plant cells, R^{memb} is usually from 1000 to 8000 ohm-cm² (see Hope).

Let us now see how large $E_{a.t.j}$ given by Equation 3.20 might be for representative electrogenic "pumps." The electrical potential drop created by active transport of 1 picomole of a monovalent cation/cm²-sec across a membrane resistance of 3000 ohm-cm² would be $(+1)(96,487 \text{ coulombs/mole})(10^{-12} \text{ mole/cm}^2\text{-sec})(3000 \text{ ohms-cm}^2)$, or 0.3×10^{-3} volt, which is a rather small potential difference. When the membrane resistance is higher

or the net flux by the electrogenic pump is greater, the potential created by the active transport process would of course be larger. For certain plant cells and some animal cells, R^{memb} is about $10,000 \text{ ohm-cm}^2$ and $J_{a.t.,j}$ may be $20 \text{ picomoles/cm}^2\text{-sec}$ (see Hope). Equation 3.20 indicates that the potential drop created by such an electrogenic pump for a monovalent cation would be approximately $(+1)(96,487)(20 \times 10^{-12})(10,000)$, or 0.019 v (19 mv). In any case, the actual electrical potential difference across a membrane is obtained by adding the potential drop caused by active transport of uncompensated charge (Eq. 3.20) and that for passive fluxes, the latter being predicted by the Goldman equation (Eq. 3.19).

Q_{10} , a temperature coefficient. In general, most metabolic reactions are markedly influenced by temperature, while processes like light absorption are insensitive to temperature. What temperature dependence should we expect for diffusion? Can we decide whether the movement of some solute into a cell is by active transport or by passive diffusion once we know how the fluxes depend on temperature? To answer such questions, we need an expression describing the distribution of energy among the molecules as a function of temperature in order to determine what fraction of the molecules has the requisite energy for a particular process. In aqueous solutions, the relevant energy is generally the kinetic energy of motion of the species involved. Hence, we will first relate the distribution of kinetic energy among molecules to temperature. At the end of this section we will present the related concept of *activation energy*—activation energies are used to discuss reactions which have an energy barrier that must be surmounted before the process can take place. The topics that we will introduce here are important for a basic understanding of many aspects of biology—all the way from biochemical reactions to the consequences of light absorption.

We begin by noting that very few molecules possess extremely large kinetic energies. In fact, the probability that a molecule has a given kinetic energy E decreases exponentially as E increases. The precise statement of this is known as the *Boltzmann energy distribution*, which describes the frequency with which specific kinetic energies are possessed by molecules at equilibrium at a given temperature (see Bull, or Edsall and Wyman). A convenient form for the Boltzmann energy distribution at temperature T is as follows:

$$n(E) = n_{\text{total}} e^{-E/kT} \quad (3.21)$$

where $n(E)$ is the number of molecules possessing an energy of E or more out of the total number of molecules, n_{total} , and k is Boltzmann's constant— $k = 8.617 \times 10^{-5} \text{ electron volts (ev)/molecule-}^\circ\text{K}$, where 1 ev is the energy

change for a particle of unit electronic charge when it moves through an electrical potential difference of 1 volt. The quantity $e^{-E/kT}$, which equals $n(E)/n_{\text{total}}$ by Equation 3.21, is often referred to as the *Boltzmann factor*. Equation 3.21 indicates that the number of molecules with an energy of zero or greater, $n(0)$, equals $n_{\text{total}} e^{-0/kT}$ or $n_{\text{total}} e^{-0}$, which is simply n_{total} , the total number of molecules present.

Due to collisions based on thermal motion, energy is continually being gained or lost by individual molecules in a random fashion. Hence a wide range of kinetic energies is possible, although very high energies are less probable, cf. Equation 3.21. As the temperature is raised, not only is the average energy per molecule higher, but the number of molecules in the "high energy tail" of the exponential Boltzmann distribution also increases substantially.

For diffusion across a membrane, the appropriate Boltzmann energy distribution indicates that the number of molecules with a kinetic energy of U or greater per molecule resulting from velocities in some particular direction is proportional to $\sqrt{T} e^{-U/kT}$ (see Davson and Danielli). A minimum kinetic energy (U_B) is often necessary to diffuse past some barrier or to cause some specific reaction. Thus, any molecule with a kinetic energy of U_B or greater has sufficient energy for the particular process. For the Boltzmann energy distribution appropriate to this case, the number of such molecules is proportional to $\sqrt{T} e^{-U_B/kT}$. (These expressions having the factor $\sqrt{T}$ actually only apply to one-dimensional cases, e.g., for molecules diffusing across a membrane.) At a temperature 10° higher, the number is proportional to $\sqrt{(T+10)} e^{-U_B/[k(T+10)]}$. The ratio of these two quantities is called the Q_{10} , or temperature coefficient of the process:

$$Q_{10} = \sqrt{\frac{T+10}{T}} e^{\frac{10U_B}{kT(T+10)}} \quad (3.22)$$

To obtain the form of the exponential given in Equation 3.22, we note that $-U_B/[k(T+10)] + U_B/(kT)$ is equal to $-TU_B/[kT(T+10)] + (T+10)U_B/[kT(T+10)]$, which is $10U_B/[kT(T+10)]$.

A Q_{10} near one is characteristic of some passive processes which have no energy barrier to surmount, i.e., where U_B equals 0. On the other hand, most enzymatic reactions take place only when the reactants have a considerable kinetic energy, so that such processes tend to be quite sensitive to temperature. A value of 2 or greater for Q_{10} is often considered to indicate the involvement of metabolism, such as occurs for active transport of a solute into a cell or organelle. However, Equation 3.22 indicates that *any* process having an appreciable energy barrier (U_B) can have a large temperature coefficient.

The value of the temperature coefficient Q_{10} for a particular process can be indicative of the minimum kinetic energy required (U_B), and vice versa. A membrane often represents an appreciable energy barrier for the diffusion of charged solutes; let us say that the U_B for passive ion movement across it is 0.50 ev/molecule. Using Equation 3.22, this would lead to a temperature coefficient for T equaling 20° of $\sqrt{(303/293)} e^{(10)(0.50)/[(8.617 \times 10^{-5})(293)(303)]}$, or $1.02 e^{0.654}$, which equals 1.96. In other words, the uptake of this particular ion would approximately double with only a 10° increase in temperature. This example indicates that a passive process can have a rather high Q_{10} if there is an appreciable energy barrier, indicating that a large temperature coefficient for ion uptake does not necessarily imply active transport.

A kinetic energy of 0.50 ev or greater is possessed by only a relatively few molecules in the Boltzmann energy distribution— $n(E) = n_{\text{total}} e^{-E/kT}$, Equation 3.21. For instance, at 20° the Boltzmann factor $e^{-E/kT}$ is $e^{-(0.50 \text{ ev})/(0.0253 \text{ ev})}$, or only 2.5×10^{-9} ($kT = 0.0253 \text{ ev}$ at 20° , App. III). As T is raised, the number of such molecules in the high energy part of the Boltzmann distribution increases greatly. Many more molecules thus have the requisite kinetic energy, U_B , and consequently can take part in the process being considered. In particular, at 30° the Boltzmann factor in Equation 3.21 becomes $e^{-(0.50)/(0.0261)}$, or 4.6×10^{-9} for a kinetic energy of 0.50 ev/molecule ($kT = 0.0261 \text{ ev}$ at 30° , App. III). Hence, the Boltzmann factor for a U_B of 0.50 ev/molecule essentially doubles for a 10° rise in temperature, consistent with our Q_{10} calculation for this case.

So far in this section we have calculated on the basis of energy *per molecule*, while in many cases it is more convenient to consider energy *per mole*. In order to change from a molecule to a mole basis, we multiply Boltzmann's constant k (energy/molecule- $^\circ\text{K}$) by Avogadro's number N (molecules/mole), which gives us the gas constant R (energy/mole- $^\circ\text{K}$), i.e., R equals kN . If U and U_B are expressed as energy per mole, we simply replace k in the Boltzmann energy distribution (Eq. 3.21) and Equation 3.22 by R . Finally, as Appendix III indicates, 1 ev/molecule is equal to 23.06 kcal/mole.

The existence of an energy barrier of height U_B is related to the concept of *activation energy*, which refers to the minimum amount of energy necessary for some reaction to take place. In the case of a membrane, we can evaluate U_B by determining how the number of molecules diffusing across the membrane varies with temperature, e.g., by invoking Equation 3.22. In fact, the kinetic energy (U_B) corresponds to the activation energy for crossing the barrier. For a chemical reaction, we can also experimentally determine how the process is influenced by temperature. If we represent the activation energy per mole by A , the rate constant for such a reaction should vary

with temperature as follows:

$$\text{rate constant} = B e^{-A/RT}$$

where B is essentially a constant.*

Equation 3.23 is referred to as the *Arrhenius equation*. It was originally proposed on experimental grounds by Arrhenius at the end of the 19th century and only subsequently interpreted theoretically (see Morris). A plot of the logarithm of the rate constant versus $1/T$ is commonly known as an *Arrhenius plot*. Hence, the slope of an Arrhenius plot is proportional to the activation energy by Equation 3.23— $\ln(\text{rate constant}) = \ln B - A/RT$. Many reactions of importance in biochemistry have large values for A and are therefore extremely sensitive to temperature. Moreover, an enzyme greatly increases the rate of the reaction which it catalyzes by reducing the value of the activation energy needed (cf. Eq. 3.23). Thus, the related concepts of activation energy and energy barrier have many applications in biology (see Morris or Stein for further discussion of activation energies).

Let us finally consider the activation energies for certain cases of diffusion. For diffusion in water at 20° , A is about 4 kcal/mole for K^+ and 5 kcal/mole for mannitol (see Stein). After replacing U_B by A and k by R in Equation 3.22, we can calculate that the appropriate Q_{10} for such diffusion would be 1.3. The activation energy for the passive efflux of K^+ from many cells is about 15 kcal/mole near 20° , which corresponds to a Q_{10} of 2.5 (see Stein). Again, we conclude that a purely passive process, such as diffusion across membranes, can have a marked temperature dependence.

Ussing-Teorell equation. We will now consider ways of distinguishing between active and passive fluxes which are more reliable than determining Q_{10} s. One of the most useful physicochemical criteria for deciding whether a particular ionic movement across a membrane is active or passive is the application of the *Ussing-Teorell*, or *flux ratio* equation. For ions moving passively, this expression shows how the ratio of the influx to the efflux depends on the internal and external concentrations of that species as well as on the electrical potential difference across the membrane. If the Ussing-Teorell equation is satisfied, active transport of the ions need not be invoked. We can readily derive this expression by considering how the influx and the efflux could each be determined experimentally, as the following arguments indicate.

* Actually, B can depend on temperature. For the one-dimensional case discussed above, the number of molecules with an energy of at least U_B is proportional to $\sqrt{T} e^{-U_B/kT}$; B would then be proportional to $\sqrt{T}$.

For measuring the unidirectional inward component, or influx of a certain ion, J_j^{in} , the plant cell or tissue can be placed in a solution containing a radioactive isotope of species j . Initially, none of the radioisotope is inside the cells, hence the internal specific activity for this isotope equals zero at the beginning of the experiment. (As for any radioisotope study, only some of the molecules of species j are radioactive. The particular fraction is known as the *specific activity*, and must be determined for both c_j^o and c_j^i in the present experiment.) Since none is inside, the initial unidirectional outward component or efflux of the radioisotope, J_j^{out} , is zero, therefore, the initial net flux (J_j) of the isotope reflects J_j^{in} . From Equation 3.15, this influx of the radioisotope can be represented by $(K_j \mu_j z_j F E_M / \Delta x) [1 / (e^{z_j F E_M / RT} - 1)] c_j^o$. After the isotope has entered the cell, some of it will start coming out. Therefore, only the initial flux will give an accurate measure of the influx of the radioisotope of species j .

Once the radioactivity has built up inside to a substantial level, we remove the radioisotope from the outside solution. The flux of the isotope will then be from inside the cells to the external solution. In this case, the specific activity for c_j^o equals zero, and c_j^i determines the net flux of the radioisotope. From Equation 3.15, this efflux of the isotope of species j differs in magnitude from the initial influx only by having the factor c_j^o replaced by $c_j^i e^{z_j F E_M / RT}$, while the quantities in the first two parentheses remain the same. The ratio of these two fluxes—each of which can be separately measured—takes on the following relatively simple form:

$$\frac{J_j^{\text{in}}}{J_j^{\text{out}}} = \frac{c_j^o}{c_j^i e^{z_j F E_M / RT}} \quad (3.24)$$

Equation 3.24 was independently derived by both Ussing and Teorell in 1949 (see Lakshminarayanaiah, Teorell, and Ussing), and is known either as the Ussing-Teorell equation or the flux ratio equation. It is strictly valid only for ions moving passively without interacting with other substances that may also be moving across the membrane. The present derivation makes use of Equation 3.15, which gives the passive flux of some charged species across a membrane in response to differences in the chemical potential of that species across the barrier. Equation 3.15 was derived by considering only one species at a time, hence, possible interactions between the fluxes of different species are not included in Equation 3.24. The Ussing-Teorell equation can thus be used to determine whether the observed influxes and effluxes are passive (i.e., responses to the chemical potentials of the ions on the two sides of a membrane), or whether additional factors such as interactions between species or active transport must be invoked. For exam-

ple, when active transport of species j into the cell is taking place, the actual J_j^{in} would be the passive unidirectional flux (i.e., the one predicted by Eq. 3.24) plus the influx due to active transport. After one further comment about Equation 3.24, we will consider a specific example of the use of the Ussing-Teorell equation to evaluate membrane fluxes.

The ratio of the influx of species j to its efflux, as given by Equation 3.24, can readily be related to the difference in its chemical potential on the two sides of the membrane, $\mu_j^o - \mu_j^i$. This difference causes the flux ratio to differ from unity, and we will return to it to estimate the minimum amount of energy needed to actively transport that ionic species across the membrane. After taking logarithms of both sides of the Ussing-Teorell equation (Eq. 3.24) and multiplying by RT , we obtain the following equalities:

$$\begin{aligned} RT \ln \frac{J_j^{\text{in}}}{J_j^{\text{out}}} &= RT \ln \frac{c_j^o}{c_j^i} - z_j F E_M \\ &= RT \ln a_j^o + z_j F E^o - RT \ln a_j^i - z_j F E^i \\ &= \mu_j^o - \mu_j^i \end{aligned} \quad (3.25)$$

where the membrane potential (E_M) has been replaced by $E^i - E^o$ in keeping with our previous convention. The derivation is restricted to the case of constant γ_j ($\gamma_j^o = \gamma_j^i$), which means that $\ln(c_j^o/c_j^i)$ equals $\ln(\gamma_j^o c_j^o/\gamma_j^i c_j^i)$, or $\ln(a_j^o/a_j^i)$, which is $\ln a_j^o - \ln a_j^i$. Finally, the $\bar{V}_j P$ term in the chemical potential is ignored for the charged species—actually, we need only assume that $\bar{V}_j P^o$ equals $\bar{V}_j P^i$ —so that μ_j then equals $\mu_j^* + RT \ln a_j + z_j F E$, where μ_j^* has the same value on the two sides of the membrane. Thus, $\mu_j^o - \mu_j^i$ is $RT \ln a_j^o + z_j F E^o - RT \ln a_j^i - z_j F E^i$, as is indicated both in Equation 3.25 and the caption to Figure 3.3.

A difference in chemical potential of species j across a membrane would cause the flux ratio of the passive fluxes to differ from unity, a conclusion that follows directly from Equation 3.25. When μ_j^o equals μ_j^i , the influx would balance the efflux, hence there would be no net passive flux of species j across the membrane ($J_j = J_j^{\text{in}} - J_j^{\text{out}}$ by Eq. 3.15). This condition ($\mu_j^o = \mu_j^i$) is also described by Equation 3.4, used to derive the Nernst equation. In fact, in such a case of equality of the chemical potentials, the electrical potential difference across the membrane is the Nernst potential, E_{N_j} , given by Equation 3.5, $E_{N_j} = (RT/z_j F) \ln(a_j^o/a_j^i)$. Thus, when E_M equals E_{N_j} for some species, J_j^{in} equals J_j^{out} , and no net passive flux of that ion is expected across the membrane nor is any energy expended in moving the ion from one side of the membrane to the other. When Equations 3.24 and 3.25 are not satisfied for some species, such ions are not moving across

the membrane passively, or perhaps not moving independently from other fluxes. One way this may occur is for the various fluxes to be interdependent, a condition describable by irreversible thermodynamics (see p. 138). Another way is through active transport of the ions, whereby energy derived from metabolism is used to move solutes to regions of higher chemical potential.

Example of active transport. The above principles for deciding whether or not active transport of certain ions is taking place can be illustrated by using data obtained with the large internodal cells of *Nitella translucens* (Table 3.1). All the parameters appearing in the Ussing-Teorell equation have been measured for Na^+ , K^+ , and Cl^- using *Nitella*. For experimental purposes, this fresh water alga is often placed in a dilute aqueous solution containing 1 mM NaCl, 0.1 mM KCl, plus 0.1 mM CaCl_2 —this solution is not unlike the pond water in which *Nitella* grows, and is generally referred to as “artificial pond water”—which indicates the values for all three c_j^o s. The concentrations of Na^+ , K^+ , and Cl^- measured in the cytoplasm, the c_j^i s, are given in Table 3.1, column 3. Assuming that activities can be replaced by concentrations, we can calculate the Nernst potential across the plasmalemma from these concentrations by using Equation 3.5, $E_{N_j} = (58.2/z_j) \log (c_j^o/c_j^i)$ in mv (the numerical factor is 58.2 since the measurements were at 20°). E_{N_j} for Na^+ is $[(58.2 \text{ mv})/(1)] \log [(1.0 \text{ mM})/(14 \text{ mM})]$, or -67 mv; for K^+ it is -179 mv; and for Cl^- the Nernst potential is 99 mv

Table 3.1
Concentrations, potentials, and fluxes of various ions for *Nitella translucens* in the light and the dark.

The superscript o refers to concentrations in the external bathing solution, superscript i refers to the cytoplasm. The Nernst potentials (E_{N_j}) were calculated from Equation 3.5 by using concentration ratios and a numerical factor of 58.2 mv since the temperature was 20°. The potential across the plasmalemma (E_M) was -138 mv. The fluxes indicated for the dark refer to values soon after removing the illumination (about 1000 lux) from the cells. A picomole is 10^{-12} mole (App. IV).

Ion	C_j^o (mM)	C_j^i (mM)	E_{N_j} (mv)	$C_j^o/C_j^i e^{z_j F E_M / RT}$	Light		Dark	
					J_j^{in}	J_j^{out}	J_j^{in}	J_j^{out}
					(picomole/cm ² -sec)			
Na ⁺	1.0	14	-67	17	0.55	0.55	0.55	0.1
K ⁺	0.1	119	-179	0.20	0.85	0.85	0.2	(0.85)
Cl ⁻	1.3	65	99	0.000085	0.85	0.85	0.05	—

SOURCE: E. A. C. MacRobbie, *Journal of General Physiology* 45: 861-878 (1962) and R. M. Spanswick and E. J. Williams, *Journal of Experimental Botany* 15: 193-200 (1964). Data reprinted by permission.

(column 4). A direct measurement of the electrical potential drop across the plasmalemma (E_M) gives -138 mv, as was indicated earlier in discussing the Goldman equation. Since E_{N_j} differs from E_M in all three cases, none of these ions is in equilibrium across the plasmalemma of *Nitella*.

A difference between E_M and E_{N_j} for a particular species indicates departure from equilibrium for that ionic species; it also tells in what compartment μ_j is higher. Specifically, if the actual membrane potential is algebraically more negative than the calculated Nernst potential, the chemical potential in the inner aqueous phase (here the cytoplasm) is lower for a cation (Fig. 3.3) but higher for an anion, compared to the values in the external solution—cf. the effect of z_j in the electrical term in the chemical potential, z_jFE . Since E_M (-138 mv) is more negative than $E_{N_{Na}}$ (-67 mv), Na^+ is at a lower

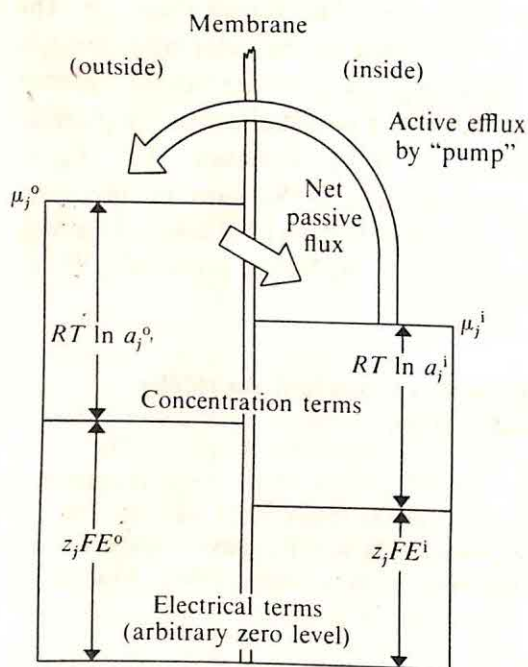


Figure 3.3

Diagram illustrating the situation for an ion not in equilibrium across a membrane.

Since μ_j^o is greater than μ_j^i , there is a net passive flux into the cell. In the steady state, this net inward flux is balanced by an equal efflux caused by active transport of species j out of the cell. We note that $\mu_j^o - \mu_j^i$ equals $z_jF(E_{N_j} - E_M)$, where $z_jFE_{N_j}$ is $RT \ln a_j^o - RT \ln a_j^i$ and $-z_jFE_M$ is $z_jFE^o - z_jFE^i$.

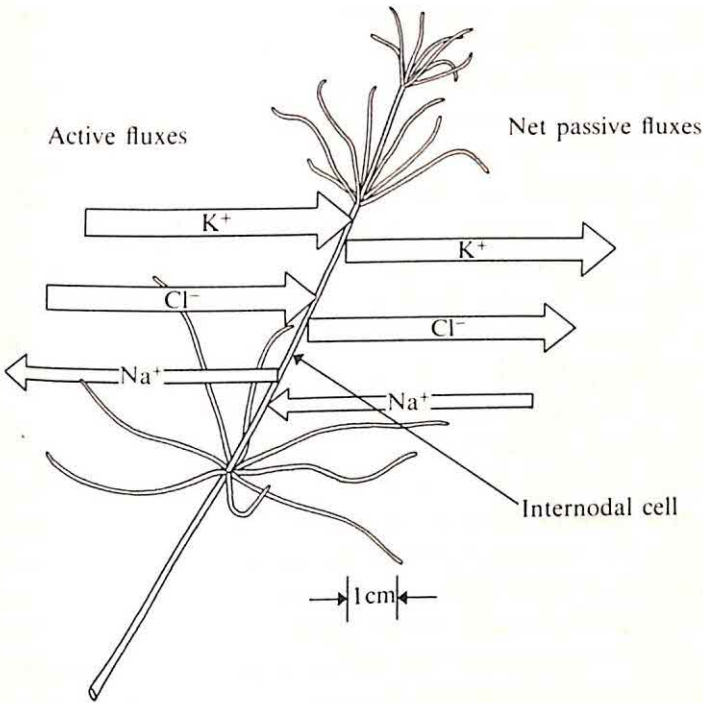


Figure 3.4

In the steady state condition in the light, the three active fluxes across the plasmalemma of the large internodal cell of *Nitella* are balanced by the net passive effluxes of potassium and chloride and the net passive influx of sodium.

chemical potential in the cytoplasm than outside in the external solution. Reasoning in an analogous manner, we find that K^+ ($E_{NK} = -179$ mv) has a higher chemical potential inside, while Cl^- ($E_{NCl} = 99$ mv) is at a much higher chemical potential inside. If these ions can move across the plasma-lemma, this suggests an active transport of K^+ and Cl^- into the cell and an active extrusion of Na^+ from the cell, as is schematically indicated in Figure 3.4.

We can also consider the movement of the various ions into and out of *Nitella* in terms of the Ussing-Teorell equation to help determine whether active transport need be invoked to explain the fluxes. The Ussing-Teorell equation predicts that the quantity appearing on the right side of Equation 3.24 ($c_j^o/c_j^i e^{z_j FEM/RT}$) should equal the ratio of influx to efflux of the various ions, if the ions are moving passively in response to gradients in their chemical potential. Using the values given in Table 3.1, this ratio (J_j^{in}/J_j^{out})

is calculated for sodium as follows: the factor RT/F is 25.3 mv at 20° (App. III), hence $e^{z_j F E_M / RT}$ is $e^{(1)(-138 \text{ mv})/(25.3 \text{ mv})}$, or $e^{-5.45}$ for an E_M of -138 mv. Therefore, $c_{\text{Na}}^o / (c_{\text{Na}}^i e^{z_{\text{Na}} F E_M / RT})$ is $(1.0 \text{ mM}) / [(14 \text{ mM})(e^{-5.45})]$, which equals 17. Following the same procedure, the expected flux ratio is calculated to be 0.20 for K^+ and 0.000085 for Cl^- (values given in Table 3.1, column 5). However, the observed influxes in the light equal the effluxes for each of these three ions (Table 3.1, columns 6 and 7). Equal influxes and effluxes are quite reasonable for mature cells of *Nitella*, which can be described by a steady-state condition. On the other hand, if J_j^{in} equals J_j^{out} , the flux ratios given by Equation 3.24 are not satisfied for Na^+ , K^+ , or Cl^- . As suggested by Figure 3.4, active transport of K^+ and Cl^- in and Na^+ out accounts for the marked deviations from the Ussing-Teorell equation for *Nitella*.

As we mentioned earlier, another approach for studying active transport is to remove the supply of energy. In the case of *Nitella*, cessation of illumination causes an appreciable decrease in the Na efflux, in the K influx, and in the Cl influx (Table 3.1, columns 8 and 9). But these are the three fluxes which are toward regions of higher chemical potential for the particular ions involved, and thus we may reasonably expect all three to be active. On the other hand, some fluxes remain essentially unchanged by placing the cells in the dark (values in Table 3.1, columns 8 and 9, refer to the fluxes soon after extinguishing the light, not the steady-state fluxes). For instance, the Na influx and K efflux are initially unchanged when the *Nitella* cells are transferred from the light to the dark, i.e., these unidirectional fluxes toward lower chemical potentials do not depend on energy derived from photosynthesis. (For reasons which are as yet unclear, the energetically downhill efflux of Cl^- apparently increases in the dark. This $J_{\text{Cl}}^{\text{out}}$ is not included in Table 3.1.)

The passive diffusion of the ions toward regions of lower chemical potential helps create the electrical potential difference across a membrane, while active transport is instrumental in maintaining the asymmetrical ionic distributions which sustain the passive fluxes. Thus, the passive and the active fluxes are interdependent in the ionic relations of cells and both are crucial for the generation of the observed diffusion potentials. Moreover, active and passive fluxes can occur simultaneously in the same direction. For example, we calculated that $J_{\text{Na}}^{\text{in}}$ should equal 17 times $J_{\text{Na}}^{\text{out}}$, if both fluxes were passive ones obeying the Ussing-Teorell equation. Since $J_{\text{Na}}^{\text{in}}$ is passive and equal to 0.55 picomole/cm²-sec, we expect a passive efflux of Na^+ equaling $(0.55)/(17)$, or 0.03 picomole/cm²-sec. Thus, the active component of the Na efflux in the light may be $0.55 - 0.03$, or 0.52 picomole/cm²-sec. At cessation of illumination, $J_{\text{Na}}^{\text{out}}$ decreases from 0.55

to 0.1 picomole/cm²-sec (Table 3.1). Extinguishing the light removes photosynthesis as a possible energy source for active transport, but respiration could still supply energy in the dark. This helps explain why $J_{\text{Na}}^{\text{out}}$ in the dark does not decrease all the way to 0.03 picomole/cm²-sec, the value predicted for the passive efflux.

Suppose that the chemical potential of some species is higher outside than inside a cell, as is illustrated in Figure 3.3. The minimum amount of energy needed to transport a mole of that species from the internal aqueous phase on one side of some membrane to the external solution on the other is the difference in chemical potential of that solute across the membrane, $\mu_j^o - \mu_j^i$ (here, $\mu_j^o > \mu_j^i$). As we noted in considering Equation 3.25, the quantity $\mu_j^o - \mu_j^i$ for ions is $RT \ln (a_j^o/a_j^i) - z_j F E_M$. Since the Nernst potential, E_{N_j} , is $(RT/z_j F) \ln (a_j^o/a_j^i)$ (Eq. 3.5), we can express the difference in chemical potential across the membrane as follows:

$$\mu_j^o - \mu_j^i = z_j F (E_{N_j} - E_M) \quad (3.26)$$

or

$$\mu_j^i - \mu_j^o = z_j F (E_M - E_{N_j})$$

We will use the bottom line of Equation 3.26 to discuss the active transport of ions *into* cells (i.e., when $\mu_j^i > \mu_j^o$).

Using the Nernst potentials of Na⁺, K⁺, and Cl⁻ for *Nitella translucens* and the value of E_M given in Table 3.1, we can calculate $z_j (E_M - E_{N_j})$ in Equation 3.26 for transporting these ions across the plasmalemma of this alga. Such a quantity is $(+1)[(-138 \text{ mv}) - (-67 \text{ mv})]$, or -71 mv for Na⁺, +41 mv for K⁺, and +237 mv for Cl⁻. These values for $z_j (E_M - E_{N_j})$ mean that Na⁺ is at a higher chemical potential in the external bathing solution while K⁺ and Cl⁻ are at higher chemical potentials inside the cell, as we concluded above (also, see Figs. 3.3 and 3.4). From Equation 3.26, the minimum energy required to actively transport, or "pump" Na⁺ out across the plasmalemma of the *Nitella* cell equals F times the energy difference in millivolts, which is $(0.02306 \text{ kcal/mole-mv})(71 \text{ mv})$, or 1.6 kcal/mole in the case of Na⁺ (F is 0.02306 kcal/mole-mv, App. II). Potassium requires $(0.02306)(41)$, or 0.9 kcal/mole to be pumped inward. The active extrusion of Na⁺ from certain algal cells may be linked to the active uptake of K⁺, ATP being implicated as the energy source for this coupled exchange process (see MacRobbie 1970 or Raven). As we will discuss later (Ch. 6), the hydrolysis of ATP under biological conditions usually releases at least 10 kcal of energy/mole. For the case of a *Nitella* cell, this is sufficient energy per mole of ATP hydrolyzed to pump a mole of Na⁺ out (1.6 kcal/mole) and

one of K^+ in (0.9 kcal/mole). The transport of Cl^- inward takes a minimum of (0.02306)(237), or 5.5 kcal/mole, according to Equation 3.26, which is a fairly large amount of energy. Neither the form of the energy used nor the actual mechanism involved in actively transporting chloride into *Nitella* or other plant cells is fully understood at the present time, although exchanges with OH^- appear to be involved.

The active uptake of K^+ and Cl^- together with an active extrusion of Na^+ in the case of *Nitella* are actually fairly widespread phenomena among plant cells (see Anderson, Gutknecht and Dainty, and MacRobbie 1971). We might ask, why does a cell actively transport K^+ and Cl^- in and Na^+ out? Although no completely satisfactory answer can be given to such a question, we shall *speculate* on possible reasons, based on the principles we have been considering.

Let us imagine that a membrane-bounded cell containing negatively charged proteins is placed in an NaCl solution, the latter possibly reflecting primeval conditions when life originated. When Na^+ and Cl^- are both in equilibrium across the membrane, E_M equals $E_{N_{Cl}}$ and $E_{N_{Na}}$. Using concentrations in Equation 3.5, $\log(c_{Na}^o/c_{Na}^i)$ then equals $-\log(c_{Cl}^o/c_{Cl}^i)$, or $c_{Na}^o/c_{Na}^i = c_{Cl}^i/c_{Cl}^o$, hence $c_{Na}^o c_{Cl}^o = c_{Na}^i c_{Cl}^i$. For electroneutrality in an external solution containing only NaCl, c_{Na}^o equals c_{Cl}^o . Since $a^2 = bc$ implies that $2a \leq b + c$, we conclude that $c_{Na}^o + c_{Cl}^o \leq c_{Na}^i + c_{Cl}^i$. But the proteins, which cannot diffuse across the membrane, also make a contribution to the internal osmotic pressure, so that π^o would be less than π^i . Water tends to flow of course toward higher osmotic pressures. When placed in an NaCl solution, water would therefore tend to enter such a membrane-bounded cell containing proteins, causing it to swell without limit. Thus, an outwardly directed active transport of Na^+ might have originated to lower c_{Na}^i and thus to prevent excessive swelling of primitive cells.

An energy-dependent uptake into a plant cell tends to increase π^i , which leads to a rise in P^i . This higher internal hydrostatic pressure favors cell enlargement and consequently cell growth. For a plant cell surrounded by a cell wall we might therefore expect an active transport of some species into the cell, e.g., chloride. (Animal cells do not have to push against a cell wall in order to enlarge, and do not generally have an active uptake of Cl^- .) Enzymes have evolved that operate efficiently when exposed to relatively high concentrations of K^+ and Cl^- . In fact, many actually require K^+ for their activity, so that an inwardly directed K pump is probably necessary for biochemistry as we know it. The presence of a substantial concentration of such ions insures that electrostatic effects adjacent to a Donnan phase could in large part be screened out—otherwise, a negatively charged substrate, e.g., an organic acid or a phosphorylated sugar, might be elec-

trostatically repelled from the catalytic site on an enzyme (proteins are generally negatively charged at cytoplasmic pH's). Once active transport has set up certain concentration differences across the membrane, the membrane potential is an inevitable consequence of the tendency of such ions to passively diffuse toward regions of lower chemical potential. Whether such diffusion potentials actually benefit the general plant cell is an open question. They are essential however for the transmission of electrical impulses in excitable cells in animals and in certain plants.

Carriers, solute uptake, and the Michaelis-Menten formalism. Although active transport is clearly of common occurrence, its actual mechanism—including the means whereby metabolic energy is used—remains uncertain. The possible involvement of a “carrier” molecule in such active transport of solutes across plant cell membranes was first suggested by W. J. V. Osterhout in the early 1930s. This carrier was proposed to bind selectively certain molecules and then to carry, or ferry them across the membrane. Carriers can provide a cell with the specificity or selectivity needed to control the entry and exit of the various types of solutes encountered. Thus, certain metabolites can be specifically taken into the cell, while photosynthetic and waste products can be selectively moved out across the membranes. Also, the active transport of certain inorganic nutrients into epidermal cells in the root allows a plant to obtain and accumulate these solutes from the soil. Even though the all-important mechanism for physically binding and moving the solutes through the membrane is still unknown, the carrier concept has found widespread application in the interpretation of experimental observations (see Bittar; Epstein; Läuger; Neame and Richards; Stein; or Yariv *et al.*).

Based primarily on results from competition studies, certain solutes are described as being bound to, or associated with a particular carrier. When an ion of some species is attached to a carrier, another similar ion (of the same or a different species) competing for the same binding site cannot also be bound. For example, the similar monovalent cations K^+ and Rb^+ appear to bind in a competitive fashion to the same carrier. For some cells, one and the same carrier might transport Na^+ out of the cell and K^+ in—the so-called sodium-potassium pump alluded to above. Calcium and strontium may compete with each other for binding sites on another common carrier. Two other divalent cations, Mg^{++} and Mn^{++} , are apparently transported by a single carrier that is different from the one for Ca^{++} and Sr^{++} . The halides (Cl^- , I^- , and Br^-) may also be transported by a single carrier. Although ions have been shown to bind specifically to certain proteins isolated from cellular membranes, much remains to be learned about carriers.

One of the most important variables in the study of carrier-mediated

uptake is the external concentration. As the external concentration of a solute increases, the rate of uptake generally reaches an upper-limiting value. We may then presume that all binding sites on the carriers for that particular ion or neutral solute have become filled or saturated. In particular, the rate of active uptake of species j , J_j^{in} , is often proportional to the external concentration of that solute (c_j^o) over the lower range of concentrations, leveling off as c_j^o is raised, and eventually approaching a maximum rate for the influx ($J_j^{\text{in}}{}_{\text{max}}$). We can describe this kind of behavior by the following equation:

$$J_j^{\text{in}} = \frac{J_j^{\text{in}}{}_{\text{max}} c_j^o}{K_j + c_j^o} \quad (3.27)$$

where K_j is a constant characteristic of species j crossing a particular membrane, expressed in the units of concentration. For the uptake of many ions into roots and other plant tissues, $J_j^{\text{in}}{}_{\text{max}}$ is 1 to 10 $\mu\text{moles/g}$ fresh weight-hr. Often, two different K_j s are observed for the uptake of one and the same ion into a root. The lower one is generally between 6 and 100 μM (which is in the concentration range of many ions in the soil water), while the other K_j can be above 10 mM (see Epstein).

Equation 3.27 is similar in appearance to the Michaelis-Menten equation used for describing enzyme kinetics in biochemistry (see Epstein; Lehninger; or White *et al.*). The substrate concentration in the latter relation is analogous to c_j^o in Equation 3.27) and the enzyme reaction velocity to J_j^{in} . The term in the Michaelis-Menten equation equivalent to K_j in Equation 3.27 is the substrate concentration for half-maximal velocity of the reaction, K_M (the Michaelis constant). We can press the analogy between enzyme kinetics and active uptake even further. The lower the K_M , the greater is the affinity of the enzyme for the substrate. Likewise, a low value for K_j indicates that the ion or other solute is more readily bound to some substance in the membrane such as a "carrier" molecule. The physiological consequence of a low K_j is that species j can be efficiently bound, even when its external concentration is relatively low; such a species is actually favored or selected for active transport into the cell.

The two most common ways of graphing data on solute uptake which fit Equation 3.27 are illustrated in Figure 3.5. Figure 3.5a shows that when the external concentration of species j , c_j^o , is equal to K_j , J_j^{in} equals $\frac{1}{2}J_j^{\text{in}}{}_{\text{max}}$, as we can see directly from Equation 3.27, i.e., J_j^{in} is then $J_j^{\text{in}}{}_{\text{max}}K_j/(K_j + K_j)$, or $\frac{1}{2}J_j^{\text{in}}{}_{\text{max}}$. Thus, K_j is the external concentration at which the rate of active uptake is half-maximal—in fact, the observed values of K_j have proved to be convenient parameters for describing the uptake of

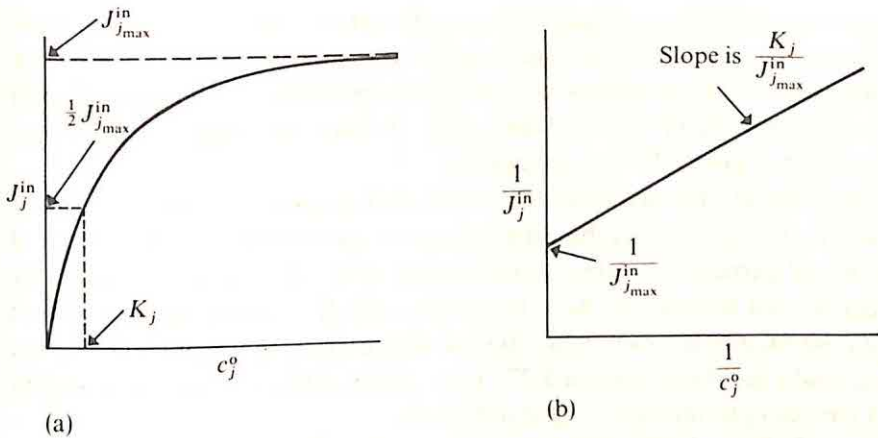


Figure 3.5

Relationship between the external solute concentration (c_j^o) and the rate of influx (J_j^{in}) for active uptake according to a Michaelis-Menten type of kinetics, as given by Equation 3.27: (a) linear plot (b) reciprocal plot.

various solutes. If two different solutes compete for the same site on some carrier, then J_j^{in} for species j will be decreased by the presence of the second solute, which is known as *competitive inhibition* of species j . As the concentration c_j^o is increased, the uptake continuously rises and eventually approaches an upper limit, $J_j^{\text{in}}_{\text{max}}$, which is the asymptotic value for the active influx (Fig. 3.5a). In the case of competitive inhibition, $J_j^{\text{in}}_{\text{max}}$ is not affected, since in principle we can raise c_j^o high enough so that the same maximum rate for the active uptake of species j is eventually obtained. However, the half-maximum rate occurs at a higher concentration, which is another way of saying that K_j is raised if a competing solute is present.

For many purposes, it is advantageous to plot the experimental data in such a way that a linear relationship is obtained when Equation 3.27 is satisfied for the active uptake of species j . Taking reciprocals of both sides of Equation 3.27, we note that $1/J_j^{\text{in}}$ equals $(K_j + c_j^o)/(J_j^{\text{in}}_{\text{max}}c_j^o)$, which is $K_j/(J_j^{\text{in}}_{\text{max}}c_j^o) + 1/J_j^{\text{in}}_{\text{max}}$. Hence, when $1/J_j^{\text{in}}$ is plotted versus $1/c_j^o$ (Fig. 3.5b), Equation 3.27 takes the form of a straight line of slope $K_j/J_j^{\text{in}}_{\text{max}}$, with an intercept on the ordinate of $1/J_j^{\text{in}}_{\text{max}}$. This latter method of treating the experimental results has proved to be rather convenient, and has found widespread application in studies of solute uptake by plant tissues, especially into roots. Since K_j increases while $J_j^{\text{in}}_{\text{max}}$ remains the same for competitive inhibition, the presence of a competing species causes the slope of the line in Figure 3.5b (i.e., $K_j/J_j^{\text{in}}_{\text{max}}$) to be greater, while the intercept on the y-axis ($1/J_j^{\text{in}}_{\text{max}}$) is unchanged. Certain compounds that are structurally unrelated

to species j can inhibit the functioning of the carriers. Such a *noncompetitive* inhibitor does not bind to the site used for transporting species j across the membrane, and K_j is unaffected. Since a noncompetitive inhibitor lowers the maximum rate of active influx ($J_j^{\text{in}}{}_{\text{max}}$), both the slope and the y -axis intercept in Figure 3.5b are changed.

The basis of the mathematical form of Equation 3.27 for describing uptake is the competitive binding of solutes to specific sites on a limited number of carriers. In other words, active processes involving metabolic energy do not necessarily have to be invoked. If a solute were to diffuse across the membrane only when bound to a carrier, the expression for the influx could also be Equation 3.27. This passive entry of a solute mediated by a carrier is termed *facilitated diffusion*.

Since facilitated diffusion is so important in biology and yet is often misunderstood, we will briefly elaborate upon it here. Certain molecules passively enter cells more readily than would be expected from consideration of their molecular structure or observations with analogous substances (e.g., by comparing P_j 's). It would appear that some mechanism is *facilitating* their entry. Moreover, the net flux is still toward lower chemical potentials and hence would be in the same direction as ordinary diffusion.* To help explain facilitated diffusion, carriers are proposed to act as shuttles for a net passive movement of the specific molecules across the membrane toward regions of lower energy. Instead of the usual diffusion across the barrier—based on random thermal motion of the solutes—carriers select out and bind certain molecules. The solute-carrier complex then diffuses across the membrane—or perhaps the solute-carrier complex simply rotates 180° in the membrane—and, finally, the solutes are somehow released on the other side of the membrane without the intervention of metabolism. Such facilitation of entry by carriers may also be regarded as a special means of lowering the activation energy needed for the solute to cross the energy barrier represented by the membrane. Thus, carriers facilitate the influx of solutes in the same way that enzymes facilitate biochemical reactions.†

There are certain general characteristics of a facilitated diffusion system. As already mentioned, the net flux is toward lower chemical potentials.

* The term "diffusion" usually refers to net thermal motion toward regions of lower concentration. It is used here in a somewhat broader context, namely, net motion toward regions of lower chemical potential.

† As with any carrier mechanism, the exact molecular details have not been worked out. Again, we are using the term "carrier" in a phenomenological or operational sense.

(According to the usual definition, p. 118, active transport is in the energetically uphill direction and, conceivably, it can employ the same carriers used in facilitated diffusion.) Movement by facilitated diffusion is more rapid than that expected for ordinary diffusion. Also, the carriers can exhibit a high degree of selectivity, i.e., they can be specific for certain types of molecules, while not binding closely related species. In addition, carriers in facilitated diffusion become saturated when the external concentration of the species transported is raised sufficiently, a behavior consistent with Equation 3.27. Finally, since carriers can exhibit competition phenomena, the flux of a species entering the cell by facilitated diffusion can be reduced when structurally analogous molecules are added to the external solution. (Such molecules would compete for sites on the carrier and thereby reduce the binding and the subsequent transfer of the original species into the cell.)

For convenience, we have been discussing facilitated diffusion *into* a cell, while exactly the same principles apply for exit. Let us assume that a carrier for potassium exists in the membrane of a certain cell and that it is being used as a shuttle for facilitated diffusion. Not only would the carrier lead to an enhanced net flux toward the side with the lower chemical potential, but also both the unidirectional fluxes J_K^{in} and K_K^{out} could be increased over the values predicted for ordinary diffusion. This increase in the unidirectional fluxes by a carrier has often been called "exchange diffusion." In such a case the molecules are interacting with a membrane component, viz., the carrier, hence the Ussing-Teorell equation—Equation 3.24, $J_j^{\text{in}}/J_j^{\text{out}} = c_j^o/(c_j^i e^{z_j FEM/RT})$ —would not be obeyed, since it presupposes that there are no interactions with other substances. In fact, observation of departures from predictions of the Ussing-Teorell equation is often the way cases of exchange diffusion are actually discovered.*

Both active and passive fluxes across the cellular membranes can occur concomitantly; these movements depend on concentrations in rather different ways. For passive diffusion, the unidirectional component J_j^{in} is proportional to c_j^o , as is indicated by Equation 1.8 for neutral solutes [$J_j = P_j(c_j^o - c_j^i)$], and by Equation 3.15 for ions. This proportionality strictly applies only over the range of external concentrations for which the permeability coefficient is essentially independent of concentration, and the membrane potential must not change in the case of charged solutes. Nevertheless,

* The term "exchange diffusion" has another usage in the literature, viz., to describe the carrier-mediated movement of some solute in one direction across a membrane in exchange for a *different* solute being transported in the opposite direction. Again, the Ussing-Teorell equation is not obeyed.

ordinary passive influxes do tend to be proportional to the external concentration, while an active influx or the special passive influx known as facilitated diffusion—either of which can be described by a Michaelis-Menten type of formalism—shows saturation effects at the higher concentrations. Moreover, facilitated diffusion and active transport exhibit both a high degree of selectivity and competition phenomena, while ordinary diffusion does not.

Principles of irreversible thermodynamics

So far, we have been using classical thermodynamics—though it may have been noticed that we have often done so somewhat illegitimately. For example, let us consider Equation 2.23 ($J_{V_w} = L_w \Delta \Psi$). Since there is a difference in chemical potential given by a change in water potential, we expect a net (and irreversible) flow of water from one region to another, which is obviously not an equilibrium situation. Strictly speaking, classical thermodynamics is concerned solely with equilibria, not with movement—classical thermodynamics might have been better-named “thermostatics”. Thus, we have frequently been concerned with a kind of hybrid—appealing to classical thermodynamics for the driving forces, and using nonthermodynamic arguments and analogies to discuss fluxes. One of the objectives of irreversible thermodynamics is to help legalize the arguments. But, as we shall see, legalizing them brings in new ideas and considerations.

Irreversible thermodynamics uses the same parameters as classical thermodynamics, viz., temperature, pressure, free energy, activity, and so on. But these quantities have been strictly defined for macroscopic amounts of matter in equilibrium situations only. How can we use them to discuss processes *not* in equilibrium, the domain of irreversible thermodynamics? This dilemma immediately circumscribes the range of validity of the theory of irreversible thermodynamics: it can only deal with “slow” processes or situations which are not very far from equilibrium, for only in such circumstances can equilibrium-related concepts such as temperature and free energy retain their validity—at least approximately. We have to assume from the outset that we can talk about and use classical thermodynamic parameters even in nonequilibrium situations.

Another refinement that we should introduce into our theory is to recognize that the movement of one species may affect the flux of a second species. A particular flow of some solute may interact with another flux by way of collisions, each species flowing under the influence of its own force. For

example, water, ions, and other solutes moving through a membrane toward regions of lower values for their respective chemical potentials can exert a frictional drag on each other. In this case, the magnitude of the flux of a solute may depend on whether water is also flowing. In this way, the fluxes of various species across a membrane become interdependent. Stated more formally, the flux of a solute is not only dependent on the negative gradient of its own chemical potential—which is the sole driving force we have recognized up to now—but it may also be influenced by the gradient in the chemical potential of water. Again using Equation 2.23 ($J_{vw} = L_w \Delta\Psi$) as an example, we have considered that the flow of water depends only on the difference in its own chemical potential between two locations, and have thus far ignored any coupling to concomitant fluxes of solutes.

A quantitative description of interdependent fluxes and forces is given by irreversible thermodynamics, a subject which treats nonequilibrium situations such as those actually occurring under biological conditions.* In this brief introduction to irreversible thermodynamics we will emphasize certain underlying principles and then derive the *reflection coefficient*, a parameter important in plant and cell physiology. In order to keep the analysis manageable, we will restrict our attention to isothermal conditions, which approximate many biological situations where fluxes of water and solutes are considered. (For further details see Dainty 1963, De Groot and Mazur, Katchalsky and Curran, Kedem and Katchalsky, or Prigogine.)

Fluxes, forces, and Onsager coefficients. In our previous discussion of fluxes, the driving force leading to the flux of species j , J_j , was the negative gradient in its chemical potential, $-\partial\mu_j/\partial x$. Irreversible thermodynamics takes a more general view, namely, that the flux of species j depends not only on $-\partial\mu_j/\partial x$, but can be affected by *any* other force occurring in the system, such as the negative gradient in the chemical potential of some other species. A particular force, X_k , can likewise influence the flux of any species. Thus, the various fluxes become interdependent, or coupled, since they can respond to changes in any of the forces. Another premise in irreversible thermodynamics is that J_j is linearly dependent on the various forces. This means that in practice we will treat only those cases which are not too far from equilibrium. Even with such a simplification the algebra often becomes rather cumbersome, owing to the coupling of the various forces and fluxes.

* Nonequilibrium and irreversible are related, since a system in a *nonequilibrium* situation left isolated from external influences will spontaneously and *irreversibly* move toward equilibrium.

Using a linear combination of all the forces, we can represent the flux of species j by

$$J_j = \sum_k L_{jk} X_k = L_{j1} X_1 + L_{j2} X_2 + \cdots + L_{jj} X_j + \cdots \quad (3.28)$$

where the summation, $\sum_k$, is over all forces (all X_k), and the L_{jk} are referred to as the *Onsager coefficients*, or the phenomenological coefficients, in this case for conductivity. The first subscript on these coefficients, e.g., j on L_{jk} , identifies the flux; the second subscript (i.e., k on L_{jk}) designates the force. Each term, $L_{jk} X_k$, is thus the partial flux of species j due to the particular force X_k . The individual Onsager coefficients in Equation 3.28 are therefore the proportionality factors indicating what contribution the force X_k makes to the flux of species j , J_j . Equation 3.28 is sometimes referred to as the *phenomenological equation*. Phenomenological equations are used to describe relations between observable quantities, without regard for explanations in terms of atoms or molecules. For instance, Ohm's law and Fick's laws are also phenomenological equations, likewise they assume linear relations between forces and fluxes.

Are there any relations between the various phenomenological coefficients? In fact, L_{jk} equals L_{kj} , which is known as the *reciprocity relation*. Such an equality of cross-coefficients was derived in 1931 by Onsager—awarded the Nobel Prize in chemistry in 1968—from statistical considerations which utilize the principle of “detailed balancing”—an argument involving microscopic reversibility, i.e., for local equilibrium, any molecular process and its reverse will be taking place at the same average rate in that region. In discursive language, the Onsager reciprocity relation means that the proportionality coefficient relating the flux of species k caused by the force on species j equals the proportionality coefficient giving the flux of j due to the force on k . (Strictly speaking, conjugate forces and fluxes must be used, as will be the case below. Also, see App. VIII.) The fact that L_{jk} equals L_{kj} can be further appreciated by considering Newton's third law; action and reaction must be equal. For example, the frictional drag exerted by a moving solvent on the solute is equal to the drag exerted by the moving solute on the solvent. The pairwise equality of cross-coefficients given by the Onsager reciprocity relation reduces the number of coefficients needed to describe the interdependence of forces and fluxes in irreversible thermodynamics and consequently leads to a simplification in solving the sets of simultaneous equations.

Water and solute flow. As a specific application of the principles just

introduced, we will consider in some detail the important coupling of water and solute flow. The driving forces for the fluxes are the negative gradients in chemical potential, which we will assume are proportional to the differences in chemical potential across some barrier, here considered to be a membrane. In particular, we will represent $-\partial\mu_j/\partial x$ by $\Delta\mu_j/\Delta x$, which is $(\mu_j^o - \mu_j^i)/\Delta x$ in the present case. (For convenience, the thickness of the barrier, Δx , will be incorporated into the coefficient multiplying $\Delta\mu_j$.) To help keep the algebra relatively simple, the development will be for a single nonelectrolyte. The fluxes are across a membrane permeable to both water (w) and the single solute (s), thereby removing the restriction in Chapter 2, where membranes permeable only to water were considered. From Equation 3.28, we can represent the fluxes as the following linear combination of the differences in chemical potential:

$$J_w = L_{ww}\Delta\mu_w + L_{ws}\Delta\mu_s \quad (3.29)$$

$$J_s = L_{sw}\Delta\mu_w + L_{ss}\Delta\mu_s \quad (3.30)$$

Equations 3.29 and 3.30 allow for the possibility that each of the fluxes may depend on the differences in both chemical potentials, $\Delta\mu_w$ and $\Delta\mu_s$. Four phenomenological coefficients are used in these two equations. By the Onsager reciprocity relation, L_{ws} equals L_{sw} . Thus, three different coefficients (L_{ww} , L_{ws} , and L_{ss}) are needed to describe the relationship of these two fluxes to the two driving forces. This is in contrast to Equation 3.6, $J_j = u_j c_j (-\partial\mu_j/\partial x)$, where each flux depends on but one force; accordingly, only two coefficients would then be involved in describing J_w and J_s .*

To obtain more convenient formulations for the fluxes of water and the solute, we generally express $\Delta\mu_w$ and $\Delta\mu_s$ in terms of the differences in the osmotic and the hydrostatic pressures, $\Delta\pi$ and ΔP —it is usually easier to measure ΔP and $\Delta\pi$ than it is to determine $\Delta\mu_w$ and $\Delta\mu_s$. This is a straightforward matter for $\Delta\mu_w$; the only possible ambiguity is in deciding the algebraic sign. For the present case, $\Delta\mu_w$ is the chemical potential of water on the outside minus that on the inside, $\mu_w^o - \mu_w^i$. From Equation 2.12 ($\mu_w = \mu_w^* - \bar{V}_w\pi + \bar{V}_wP + m_wgh$), $\Delta\mu_w$ is given by

$$\Delta\mu_w = -\bar{V}_w\Delta\pi + \bar{V}_w\Delta P \quad (3.31)$$

* If the solute were a salt dissociable into two ions, we would have to consider three components and three forces, $\Delta\mu_w$, $\Delta\mu_+$, and $\Delta\mu_-$. This would lead to nine coefficients in the three flux equations (for J_w , J_+ , and J_-). By invoking the Onsager reciprocity relation, we would then have six different phenomenological coefficients to describe the movement of water, a cation, and its accompanying anion.

where $\Delta\pi$ here equals $\pi^o - \pi^i$ and ΔP is $P^o - P^i$ ($\Delta h = 0$ across a membrane).

To express $\Delta\mu_s$ in terms of $\Delta\pi$ and ΔP , we must first consider the activity term, $RT \ln a_s$, in a little detail. We will begin with the differential $RT d(\ln a_s)$, which equals $RT da_s/a_s$, or $RT d(\gamma_s c_s)/\gamma_s c_s$. When γ_s is constant, this latter quantity becomes simply $RT dc_s/c_s$. From Equation 2.10 ($\pi_s = RT \sum_j c_j$), $RT dc_s$ equals $d\pi$ for a dilute solution of a single solute.

Hence, $RT d(\ln a_s)$ can be replaced by $d\pi/c_s$ as a useful approximation. In expressing the difference in chemical potential across a membrane we are interested in macroscopic changes, not in the infinitesimal changes given by differentials. To go from differentials to differences (Δ), $RT d(\ln a_s)$ becomes $RT \Delta \ln a_s$ and $d\pi/c_s$ can be replaced by $\Delta\pi/\bar{c}_s$, where $\bar{c}_s$ is essentially the mean concentration of solute s , in this case across the membrane. (Alternatively, we can simply define $\bar{c}_s$ as that concentration for which $RT \Delta \ln a_s$ exactly equals $\Delta\pi/\bar{c}_s$.) Therefore, we can replace the term $RT \Delta \ln a_s$ in $\Delta\mu_s$ by the equivalent term, $\Delta\pi/\bar{c}_s$. From Equation 2.4, μ_s equals $\mu_s^* + RT \ln a_s + \bar{V}_s P$ for a neutral species, and the difference in chemical potential of the neutral solute across the membrane ($\Delta\mu_s$) becomes

$$\Delta\mu_s = \frac{1}{\bar{c}_s} \Delta\pi + \bar{V}_s \Delta P \quad (3.32)$$

The two expressions representing the driving forces, $\Delta\mu_w$ (Eq. 3.31) and $\Delta\mu_s$ (Eq. 3.32), are expressed as functions of the same two pressure differences, $\Delta\pi$ and ΔP , the latter being rather convenient from the point of view of experiment.

Volume flow, L_p , and σ . Now that we have appropriately expressed the chemical potential differences of water and the solute, we will direct our attention to the fluxes. In our usual units, the fluxes J_w and J_s are the moles of water and solute, respectively, moving across one cm^2 of membrane surface in a second. A quantity of considerable interest in plant and cell physiology is the volume flow, J_v , which is the rate of movement of the total volume of both water and solute across unit area of the membrane. J_v has the units of volume per unit area per unit time, e.g., $\text{cm}^3/(\text{cm}^2\text{-sec})$, which is cm/sec .

The molar flux of some species j (J_j) in moles/ $\text{cm}^2\text{-sec}$ times the volume occupied by each mole of it ($\bar{V}_j$) in cm^3/mole gives the volume flow for that component (J_{V_j}) in cm/sec . For solute and water both moving across the membrane, the total volume flow J_v is the volume flow of water plus

that of solute, which in the case of a single solute can be represented as follows:

$$J_V = \bar{V}_w J_w + \bar{V}_s J_s \quad (3.33)$$

It is generally simpler and more convenient to measure the total volume flux (such as that given in Eq. 3.33) than one of the component volume fluxes ($J_{V_i} = \bar{V}_i J_i$). For instance, we can often determine the volumes of cells or organelles under different conditions and relate any changes in volume to J_V . The volume flux of water, J_{V_w} (used in Ch. 2, Eq. 2.23) is simply $\bar{V}_w J_w$.

However straightforward it may be, the algebraic substitution necessary to incorporate the various forces and fluxes into the volume flow given by Equation 3.33 leads to a rather cumbersome expression. Nevertheless, it provides—albeit in symbols—insight into the origin of the reflection coefficient, an important quantity that we will consider in detail below. When the fluxes of water and solute given by Equations 3.29 and 3.30, respectively, are substituted into the expression for the volume flow (Eq. 3.33), we obtain the following relation:

$$J_V = (\bar{V}_w L_{ww} + \bar{V}_s L_{sw}) \Delta\mu_w + (\bar{V}_w L_{ws} + \bar{V}_s L_{ss}) \Delta\mu_s \quad (3.34)$$

As we mentioned above, L_{sw} equals L_{ws} , so that only the symbol L_{ws} need be retained here. Next, we will incorporate the chemical potential differences across the membrane for water and the solute, as given by Equations 3.31 and 3.32, respectively, into Equation 3.34. Upon rearrangement, J_V is then as follows:

$$J_V = (\bar{V}_w^2 L_{ww} + 2\bar{V}_s \bar{V}_w L_{ws} + \bar{V}_s^2 L_{ss}) \Delta P - (\bar{V}_w^2 L_{ww} - \bar{V}_w \frac{1}{\bar{c}_s} L_{ws} - \bar{V}_s \frac{1}{\bar{c}_s} L_{ss} + \bar{V}_s \bar{V}_w L_{ws}) \Delta\pi \quad (3.35)$$

Equation 3.35 gives the dependence of the total volume flow on the hydrostatic and osmotic pressure differences across some membrane permeable to both water and the solute.

The quantities multiplying ΔP and $\Delta\pi$ in Equation 3.35 are rather cumbersome, so we will find it convenient to define some new parameters. The factor multiplying the difference in hydrostatic pressure, ΔP , is called the *hydraulic conductivity coefficient*, L_P . From Equation 3.35, this hydraulic conductivity coefficient has the following value:

$$L_P = \bar{V}_w^2 L_{ww} + 2\bar{V}_s \bar{V}_w L_{ws} + \bar{V}_s^2 L_{ss} \quad (3.36)$$

Such a coefficient describes the membrane conductivity, since the larger L_P ,

the greater is J_V for a given hydrostatic pressure difference across the membrane. The other unwieldy factor appearing in Equation 3.35, viz., the parenthesis multiplying $\Delta\pi$, is incorporated into the reflection coefficient, σ . It is advantageous to define σ as the ratio of the coefficient of $\Delta\pi$ divided by the coefficient of ΔP in the expression for the volume flow (Eq. 3.35). Using the definition of L_P (Eq. 3.36), σ is therefore given by

$$\sigma = \frac{\bar{V}_w^2 L_{ww} - \bar{V}_w \frac{1}{\bar{c}_s} L_{ws} - \bar{V}_s \frac{1}{\bar{c}_s} L_{ss} + \bar{V}_s \bar{V}_w L_{ws}}{L_P} \quad (3.37)$$

The reflection coefficient defined by Equation 3.37 is perhaps the most important parameter from irreversible thermodynamics relevant to the fluxes generally considered in plant physiology. It was introduced by Staverman in 1948 and specifically applied to biological situations by Kedem and Katchalsky in 1958 (see De Groot and Mazur; Katchalsky and Curran; Kedem and Katchalsky, or Prigogine for a more detailed development of the theory). Since 1958, numerous applications of irreversible thermodynamics, in general, and reflection coefficients, in particular, have been made in biology. We will shortly consider specific values of σ for solutes entering chloroplasts. We will also show that the maximum value of the reflection coefficient occurs when the solute is unable to penetrate the membrane—the molecules are then all *reflected* from the barrier. In the absence of a hydrostatic pressure difference across a membrane, the volume flow (J_V) equals $-L_P \sigma \Delta\pi$ by Equation 3.35. Thus, the magnitude of the dimensionless parameter σ determines the volume flow expected in response to a difference in osmotic pressure across a membrane—it is this use which is most pertinent in plant physiology. After further considering the expression for volume flow, we will discuss the properties of reflection coefficients. (σ is derived in a more conventional, although less intuitive way in App. VIII.)

Using L_P and σ as defined by Equations 3.36 and 3.37, respectively, we can rewrite the expression for the total volume flow (Eq. 3.35) in the following convenient form:

$$J_V = L_P(\Delta P - \sigma \Delta\pi) \quad (3.38)$$

Equation 3.38 indicates that the osmotic contribution to the volume flow across the membrane is changed by the factor σ , which is less than one when the solute can cross the membrane (as we will soon derive).

Many different solutes can penetrate a biological membrane under usual conditions. Each such species j can be characterized by its own reflection

coefficient, σ_j , for that particular membrane. The volume flow given by Equation 3.38 can then be generalized as

$$J_v = L_P(\Delta P - \sum_j \sigma_j \Delta \pi_j) \quad (3.39)$$

where $\Delta \pi_j$ is the osmotic pressure difference across the membrane for species j (e.g., $\Delta \pi_j = RT\Delta c_j$ by Eq. 2.10). Although interactions with water are still taken into account, the generalization represented by Equation 3.39 introduces the assumption that the solutes do not interact with each other as they penetrate the membrane. Moreover, J_v in Equation 3.39 refers only to the movement of neutral species—otherwise we would also need a current equation to describe the flow of charge. Nevertheless, Equation 3.39 is a useful approximation of the actual situation, describing the multicomponent solutions encountered by cells, and we will use it as the starting point for our general consideration of solute movement across membranes. Before discussing such movement, however, let us consider the range of value of reflection coefficients.

Values of reflection coefficients. A reflection coefficient characterizes some particular solute interacting with a specific membrane. In addition, σ_j depends on the solvent on either side of the membrane, as the subscripts w for water in Equation 3.37 clearly indicate—water is the only such solvent we will consider here. Two extreme conditions can describe the passage of solutes: impermeability, which leads to the maximum value of unity for the reflection coefficient, and nonselectivity, where σ_j is zero. A reflection coefficient of zero may describe the movement of a solute across a very coarse barrier (one with large pores) which does not distinguish or select between solute and solvent molecules, or it may refer to the passage through a membrane of a molecule very similar in size and structure to water itself.

Impermeability describes the limiting case where water can cross some membrane, but the solute cannot. When the solute does not penetrate the membrane at all, J_s must remain zero for any and all values of $\Delta \mu_w$ and $\Delta \mu_s$. Since J_s equals $L_{sw}\Delta \mu_w + L_{ss}\Delta \mu_s$ (Eq. 3.30), the only way in which J_s will always be zero is for both L_{ss} and L_{ws} to be zero. Therefore, when the membrane is impermeable to the particular solute under consideration, both L_{ss} and L_{ws} are zero. Putting these zero values for L_{ss} and L_{ws} into Equations 3.36 and 3.37, we obtain the following two relations: $L_P = \bar{V}_w^2 L_{ww}$ (Eq. 3.36) and $\sigma = \bar{V}_w^2 L_{ww} / L_P$ (Eq. 3.37). Consequently, σ then equals $\bar{V}_w^2 L_{ww} / \bar{V}_w^2 L_{ww}$, which is one. A reflection coefficient of unity thus signifies that the solute is not crossing the membrane, i.e., all of the solute is “re-

flected" from the membrane, and the reflection coefficient has its maximum value. When a solute can penetrate, L_{ss} takes on a positive value, i.e., the force on solute s causes a flux of it across the membrane. The $-L_{ss}\bar{V}_s/\bar{c}_s$ term then causes the numerator of the expression for σ (Eq. 3.37) to decrease as L_{ss} increases, while the denominator (L_P , see Eq. 3.36) tends to increase as the solute's ability to penetrate goes up. The reflection coefficient thus becomes less than one when the solute can cross the membrane. This last comment emphasizes the fact that the reflection coefficient defined by Equation 3.37 handles the general case of solute permeability. Moreover, the two terms containing L_{ws} in the numerator of the expression for the reflection coefficient (Eq. 3.37) take into account the further possibility that solute and solvent may interact when crossing the membrane, i.e., σ can also be affected by coupling or interdependence of forces and fluxes.

When a membrane or barrier is nonselective, both water and the solute move across it at the same velocity, i.e., $v_s = v_w$. The solution is then the same on both sides of the barrier, so that $\Delta\pi$ across the barrier is zero, which means that $\Delta\mu_w$ equals $\bar{V}_w\Delta P$ and $\Delta\mu_s$ is $\bar{V}_s\Delta P$. (Admittedly, the idea of a solution containing a single solute is not realistic from a biological point of view, but it is convenient for illustrating the minimum value for σ .) As we discussed above (p. 103), v_j is simply J_j/c_j . Using these various conditions as well as Equations 3.29 and 3.30, we obtain the following string of equalities: $v_w = J_w/c_w = L_{ww}\Delta\mu_w/c_w + L_{ws}\Delta\mu_s/c_w = (L_{ww}\bar{V}_w/c_w + L_{ws}\bar{V}_s/c_w)\Delta P = v_s = J_s/c_s = L_{ws}\Delta\mu_w/c_s + L_{ss}\Delta\mu_s/c_s = (L_{ws}\bar{V}_w/c_s + L_{ss}\bar{V}_s/c_s)\Delta P$. Next, we will let the number of moles of water per unit volume (c_w) times the volume per mole of water ($\bar{V}_w$) equal unity— $c_w\bar{V}_w \cong 1$ is another way of stating the dilute solution approximation. Also, we will identify c_s with $\bar{c}_s$. Putting these last two relations into the factors multiplying ΔP in the above string of equalities yields $L_{ww}\bar{V}_w^2 + L_{ws}\bar{V}_s\bar{V}_w = L_{ws}\bar{V}_w/\bar{c}_s + L_{ss}\bar{V}_s/\bar{c}_s$. Finally, we can use this desired relation to evaluate the numerator of Equation 3.37. Accordingly, σ equals $0/L_P$, which is zero, i.e., for the case of nonselectivity ($v_s = v_w$), the reflection coefficient of the solute must be zero for that barrier.

Another relatively simple condition by which σ equals zero can be readily understood from Equation 3.38 [$J_v = L_P(\Delta P - \sigma\Delta\pi)$]. Let us consider the rather realistic situation where ΔP is zero across a membrane. The volume flow (J_v) is then simply $-L_P\sigma\Delta\pi$, by Equation 3.38. For a solute having a reflection coefficient equal to zero for that particular membrane, the volume flow would be zero. By Equation 3.33 ($J_v = \bar{V}_wJ_w + \bar{V}_sJ_s$), a zero J_v implies that $\bar{V}_wJ_w$ equals $-\bar{V}_sJ_s$. In words, the volume flow of water must be equal and opposite to the volume flow of the solute in order to

get no net volume flow. It follows that when ΔP is zero but $\Delta\pi$ is nonzero, the absence of a net volume flow J_v across a membrane permeable to both water and the single solute indicates that the reflection coefficient for that solute is zero. This condition of σ equaling zero occurs when the volume of water flowing toward the side with the higher π is balanced by an equal volume of solute diffusing across the membrane in the opposite direction toward the side where the solute is less concentrated (lower π). Such a situation of zero volume flow anticipates the concept of a "stationary state," introduced in the next section.

Solutes interacting with biological membranes exhibit properties ranging from impermeability ($\sigma_j = 1$) all the way to nonselectivity ($\sigma_j = 0$). Substances which are retained in, or excluded from plant cells have reflection coefficients rather close to unity for the cellular membranes. For instance, the σ_j s for sucrose and amino acids are usually near 1.0 for plant cells. Methanol and ethanol enter cells very readily and have reflection coefficients of approximately 0.3 for some *Chara* and *Nitella* internodal cells (see Dainty and Ginzburg). On the other hand, σ_j can essentially equal zero for solutes crossing porous barriers, such as those presented by cell walls, or for molecules that are penetrating very readily across membranes, such as D_2O . As is the case for a permeability coefficient, the reflection coefficient of some species is the same for traversal in either direction across a membrane.

For many small neutral solutes not interacting with carriers in the membrane, the reflection coefficients are correlated with the partition coefficients (see Diamond and Wright, or Wright and Diamond). For example, when K_j for nonelectrolytes is less than about 10^{-3} , σ_j is generally close to one. Thus compounds which do not readily enter the lipid phase of the membrane (low K_j) also do not cross the membrane easily (σ_j near 1). When the partition coefficient is one or greater, the solutes can enter the membrane in appreciable amounts, and σ_j is generally close to zero. Considering the intermediate case, the reflection coefficient of some species j can be near 0.5 for a small nonelectrolyte having a K_j of about 0.1 for the membrane lipids, although individual molecules differ depending on their molecular weight, branching, and atomic composition. Neglecting frictional effects with other solutes, we see that the intermolecular interactions affecting the partition coefficient are similar to those governing the value of the reflection coefficient, and there is somewhat of a correlation therefore between the permeability coefficient of a solute ($P_j = D_j K_j / \Delta x$) and its reflection coefficient for the same membrane. In fact, Figure 3.6 illustrates that there is even a correlation between the reflection coefficients of a series of nonelectrolytes determined with animal membranes and the permeability co-

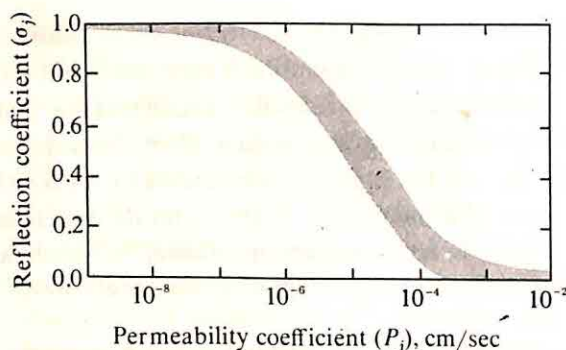


Figure 3.6

Correlation between the reflection coefficients for a series of nonelectrolytes (determined using rabbit gallbladder epithelium) and the permeability coefficients for the same compounds (measured by R. Collander using *Nitella mucronata*). Most of the measurements are in the area indicated. (The curve is adapted from J. M. Diamond and E. M. Wright, *Annual Review of Physiology* **31**: 581-646 (1969). Used by permission.)

efficients of the same substances measured for plant membranes. Thus, as the permeability coefficient goes from very small to very large values, the reflection coefficient decreases from unity (describing relative impermeability) to zero (for the opposite extreme of nonselectivity). In the next section we will specifically consider some of the consequences of reflection coefficients differing from unity for solutes crossing cellular and organelle membranes.

Solute movement across membranes

We can profitably reexamine certain aspects of the movement of solutes into and out of cells and organelles by using the more general equations developed from irreversible thermodynamics. One particularly important situation amenable to rather uncomplicated analysis is when the total volume flow J_V is zero, an example of a *stationary state*. This stationary state, in which the volume of the cell or organelle does not change over the time period of interest, can be brought about by having the net volume flow of water in one direction across the membrane equal the net volume flow of solutes

in the opposite direction. A stationary state is therefore not the same as a steady state or an equilibrium condition for the cell or organelle; it represents a situation occurring only at a particular time or under some special experimental arrangement. In this regard we should point out that μ_j can depend on both position and time for a stationary state, but only on position for a steady state, and on neither position nor time at equilibrium. When water is moving into a cell, $\Delta\mu_w$ is non-zero (we are not at equilibrium) and, in general, μ_w^i will be increasing with time (we are not even in a steady state). If over some time interval the volume flow of solutes out of the cell equals this J_{V_w} in, the cell will be in a *stationary state*, where its volume is not changing. Our restriction here to cases of zero volume flow considerably simplifies the algebra and emphasizes the role played by reflection coefficients. Moreover, a stationary state of no volume change often characterizes the experimental situation under which the Boyle-Van't Hoff relation (Eqs. 2.15 and 2.18) or the expression describing incipient plasmolysis (Eq. 2.19) is invoked. Thus, the derivation of both of these relationships will be reconsidered in terms of irreversible thermodynamics, and we will discuss the role of reflection coefficients.

What is the relationship between the internal and the external P s and π s in the case of a stationary state? In order to answer this, we must first express the consequences of a stationary state in symbols. Our point of departure is Equation 3.39, $J_V = L_P(\Delta P - \sum_j \sigma_j \Delta\pi_j)$, where the stationary state condition of zero net volume flow ($J_V = 0$) leads to the following equalities: $0 = \Delta P - \sum_j \sigma_j \Delta\pi_j = P^o - P^i - \sum_j \sigma_j (\pi_j^o - \pi_j^i)$. Here, the os-

motonic pressures are for the effect of solutes on water activity, while in the general case both osmotic contributions from solutes (π_s) and matric pressures resulting from the presence of interfaces (τ) might occur. Volume measurements for osmotic studies involving incipient plasmolysis or the Boyle-Van't Hoff relation are generally made when the external solution is at atmospheric pressure ($P^o = 0$) and when there are no external interfaces ($\tau^o = 0$). The stationary state condition of J_V equaling zero then leads to

$$\sum_j \sigma_j \pi_j^o = \sum_j \sigma_j \pi_j^i + \tau^i - P^i \quad (3.40)$$

where the possibilities of interfacial interactions and hydrostatic pressures within the cell or organelle are explicitly recognized by the inclusion of τ^i and P^i . Equation 3.40 describes the stationary state of zero volume flow.

It applies when the external solution is at atmospheric pressure (and $\tau^0 = 0$) and when the solutes are capable of crossing the barrier, as occurs for molecules interacting with real, not idealized, biological membranes.

The influence of reflection coefficients on incipient plasmolysis. In Chapter 2 we used classical thermodynamics to derive the condition for incipient plasmolysis ($\pi^0_{\text{plasmolysis}} = \pi^i$, Eq. 2.19), which occurs when the internal pressure P^i inside a plant cell just becomes zero. This derivation was made assuming equilibrium of water, i.e., equal water potential, across a membrane impermeable to solutes. The assumptions of water equilibrium and impermeability are often not valid. Can we rectify the situation using an approach based on irreversible thermodynamics?

Measurements of incipient plasmolysis can be made when there is zero volume flow ($J_v = 0$) and for a simple external solution ($\tau^0 = 0$) at atmospheric pressure ($P^0 = 0$). In this case, Equation 3.40 ($\sum_j \sigma_j \pi_j^0 = \sum_j \sigma_j \pi_j^i + \tau^i - P^i$) would be the appropriate underlying expression from irreversible thermodynamics, instead of the less realistic condition of water equilibrium previously used. Using this stationary state condition, we obtain the following expression describing incipient plasmolysis ($P^i = 0$) when the solutes can cross the cell membrane:

$$\sigma^0 \pi^0_{\text{plasmolysis}} = \sum_j \sigma_j \pi_j^i + \tau^i \quad (3.41)$$

where $\sum_j \sigma_j \pi_j^0$ has been replaced by $\sigma^0 \pi^0$. In other words, it is often convenient to characterize the external solutes by an average reflection coefficient, σ^0 , where $\sum_j \sigma_j \pi_j^0$ equals $\sigma^0 \pi^0$ and π^0 is the total external osmotic pressure, i.e., $\pi^0 = \sum_j \pi_j^0$.

Since the value of σ^0 depends on the particular external solutes present, Equation 3.41 indicates that the external osmotic pressure (π^0) at incipient plasmolysis varies from solute to solute placed in the solution surrounding the plant cells. Let us suppose that solute i cannot penetrate the membrane, so that σ_i equals 1, a situation often true for sucrose. Another solute, j , can penetrate into the cells ($\sigma_j < 1$), as is the case for many small nonelectrolytes. If we are at the point of incipient plasmolysis for each of these two solutes—taken in turn as the sole species in the external solution— $\sigma_i \pi_i^0_{\text{plasmolysis}}$ equals $\sigma_j \pi_j^0_{\text{plasmolysis}}$ by Equation 3.41. But solute i is unable

to penetrate the membrane ($\sigma_i = 1$). Hence we obtain the following relationships:

$$\sigma_j = \frac{\pi_i^{\circ} \text{ plasmolysis}}{\pi_j^{\circ} \text{ plasmolysis}} = \frac{\text{effective osmotic pressure of species } j}{\text{actual osmotic pressure of species } j} \quad (3.42)$$

Equation 3.42 suggests a rather straightforward way of describing σ_j . Since (by supposition) species j can cross the cell membrane, σ_j must be less than one. Therefore, by Equation 3.42, $\pi_j^{\circ} \text{ plasmolysis}$ is greater than $\pi_i^{\circ} \text{ plasmolysis}$, where the latter refers to the osmotic pressure of the impermeable solute at the point of incipient plasmolysis. In words, a higher external osmotic pressure is needed to cause plasmolysis if that solute is able to enter the plant cell. The "actual osmotic pressure" indicated in Equation 3.42 is defined either by Equation 2.7, $\pi = -(RT/\bar{V}_w) \ln a_w$, where $\pi = \sum_j \pi_j$, or

by Equation 2.10, $\pi_j = RT \gamma_j c_j$, when activity coefficients are included. When the membrane is impermeable to the solute, σ_j equals one, and the apparent (effective) osmotic pressure of species j equals the actual π_j° . Effective osmotic pressure takes into consideration the fact that most solutes can move across biological membranes ($\sigma_j < 1$), hence the $\Delta\pi_j$ effective in leading to a net volume flow is reduced from its actual value—cf. Equation 3.39, $J_v = L_p(\Delta P - \sum_j \sigma_j \Delta\pi_j)$. In summary, the reflection coefficient of species

j indicates how effectively the osmotic pressure of that solute can be exerted across a particular membrane or other barrier.

We can also use the condition of incipient plasmolysis described by Equation 3.42 to evaluate specific reflection coefficients. It is difficult in experiments to replace one external solution by another with no changes in the tissue taking place, or with none of the previous solution adhering to the cell (see Dainty 1963). Also, although it is easy in principle and involves only the use of a light microscope, determination of when the plasmalemma just begins to pull away from the cell wall is a rather subjective judgment. Nevertheless, the use of Equation 3.42 provides a simple way of considering individual reflection coefficients for various solutes entering plant cells.

Since osmotic pressures play a key role in plant physiology, σ_j s are important parameters for quantitatively describing the solute and water relations of plants. In particular, the incorporation of reflection coefficients allows the role of osmotic pressure to be precisely stated. In Poiseuille's law, $J_v = -(r^2/8\eta) \partial P/\partial x$ (Eq. 8.9), which can adequately describe movement in the xylem and possibly in the phloem, the flow is driven by the hydrostatic

pressure gradient. An alternative view of the same situation is that σ equals zero for the solutes. In that case, osmotic pressures would have no direct effect on the movement described by Poiseuille's law. The real interest in reflection coefficients comes when σ_j is not at one of its two extremes of zero and unity. For such cases—intermediate between nonselectivity and impermeability—the volume flow does not depend on the full osmotic pressure difference across the barrier, but it would be invalid to completely ignore the osmotic contribution of species j . (In App. VIII, an expression involving reflection coefficients is developed for the flux of a solute, J_s .)

To illustrate some of the points we have been making concerning incipient plasmolysis and reflection coefficients, let us consider Figure 3.7. The cell

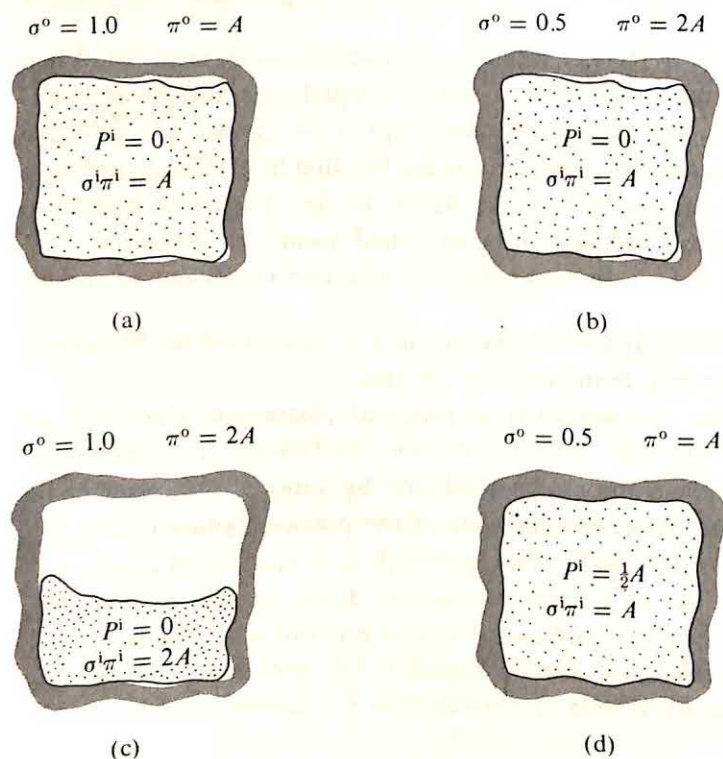


Figure 3.7

Diagrammatic cell showing a cell wall (shaded region) and a plasmalemma (thin line) for various external osmotic pressures: (a) point of incipient plasmolysis in the presence of an impermeable solute (b) point of incipient plasmolysis with a permeating solute (c) extensive plasmolysis (we are ignoring any effect of plasmodesmata) (d) cell under turgor.

in the upper left of the diagram is at the point of incipient plasmolysis ($P^i = 0$) for a nonpenetrating solute ($\sigma^o = 1.0$) in the external solution ($\pi^o = A$, where A is a constant), i.e., the plasmalemma is just beginning to pull away from the cell wall. Equation 3.41 indicates that $\sigma^i\pi^i$ would then also equal A . If we place the cell in a second solution containing a penetrating solute ($\sigma^o < 1$), Equation 3.42 indicates that the external solution must have a higher osmotic pressure in order for the cell to remain at the point of incipient plasmolysis. For instance, for a second solute with a σ_j of 0.5, the external osmotic pressure at the point of incipient plasmolysis would be $2A$ (cf. Fig. 3.7b). Thus, when the external solute can penetrate, π^o is not as effective in balancing the internal osmotic pressure or in leading to a flow of water (cf. Eq. 3.42).

On the other hand, if π^o were $2A$ for an impermeable external solute, extensive plasmolysis of the same cell would occur, as is illustrated in Figure 3.7c. Since $\sigma^o\pi^o$ is $2A$ in this case, Equation 3.40 ($\sigma^o\pi^o = \sigma^i\pi^i - P^i$, when $\tau^i = 0$) indicates that $\sigma^i\pi^i$ must also be $2A$, which means that essentially half of the internal water has left the cell. Finally, if the reflection coefficient were 0.5 and the external osmotic pressure were A , $\sigma^o\pi^o$ would be $\frac{1}{2}A$, and we would not be at the point of incipient plasmolysis. In fact, the cell would be under turgor with an internal hydrostatic pressure equal to $\frac{1}{2}A$, at least until the concentration of the penetrating solute began to build up inside. We must therefore take into account the reflection coefficients of external (and internal) solutes in order to describe conditions at the point of incipient plasmolysis and, by extension, predict the direction and the magnitude of volume fluxes across membranes.

Extension of the Boyle-Van't Hoff relation. In Chapter 2 we derived the Boyle-Van't Hoff relation assuming that the water potential was the same on both sides of the cellular or organelle membrane under consideration. Not only were equilibrium conditions imposed on water, but we implicitly assumed that the membrane was impermeable to the solutes. However, zero total volume flow ($J_v = 0$) is a better description of the experimental situation where the Boyle-Van't Hoff relation is actually applied. This condition of no volume change during the measurement is another example of a stationary state, so the Boyle-Van't Hoff relation will be reexamined from the point of view of irreversible thermodynamics. In this way, we can remove two of the previous restrictions, equilibrium for water and impermeability of solutes (see Nobel).

When molecules can cross the membranes bounding cells or organelles, the reflection coefficients for both internal and external solutes should be included in the Boyle-Van't Hoff relation. Since σ^o is less than one when

the external solutes can penetrate, the effect of the external osmotic pressure on J_v is reduced. Likewise, the reflection coefficients for solutes within the cell or organelle will lessen the contribution of the internal osmotic pressure of each solute. Replacing $\sum_j \sigma_j \pi_j^o$ by $\sigma^o \pi^o$ and π_j^i by $RT \gamma_j^i n_j^i / (\bar{V}_w n_w^i)$ (Eq. 2.10), Equation 3.40 leads to the following Boyle-Van't Hoff relation for the stationary state condition ($J_v = 0$ in Eq. 3.39):

$$\pi^o = RT \frac{\sum_j \sigma_j \gamma_j^i n_j^i}{\sigma^o \bar{V}_w n_w^i} + \frac{\tau^i - P^i}{\sigma^o} \quad (3.43)$$

The reflection coefficients of the membrane for both internal and external solutes enter into this extension of the expression relating volume and external osmotic pressure. As indicated in Chapter 2, the quantity $V - b$ in the conventional Boyle-Van't Hoff relation, $\pi^o(V - b) = RT \sum_j \varphi_j n_j$ (Eq. 2.15), can be identified with $\bar{V}_w n_w^i$. In comparing Equation 2.15 with Equation 3.43, it is evident that the osmotic coefficient of species j , φ_j , can be equated to $\sigma_j \gamma_j^i / \sigma^o$ as an explicit recognition of the activity coefficient of species j and the permeation properties of solutes, both internal and external. In fact, failure to recognize the effect of reflection coefficients on φ_j has led to misunderstandings on osmotic responses.

As we discussed in Chapter 2, the volume of pea chloroplasts responds linearly to $1/\pi^o$ (Fig. 2.5), indicating that $\tau^i - P^i$ in such plastids may be negligible compared with the external osmotic pressures used. To analyze experimental observations, it is convenient to replace $\sigma^o \pi^o$ by $\sigma_x \pi_x^o + \alpha$, where π_x^o is the contribution to the external osmotic pressure of solute x whose reflection coefficient (σ_x) is being considered, and α is the sum of $\sigma_j \pi_j^o$ for all the other external solutes. We can represent $RT \sum_j \sigma_j \gamma_j^i n_j^i$ by β , and replace $\bar{V}_w n_w^i$ by $V - b$. Making these substitutions into Equation 3.43, we obtain the following relatively simple form for the Boyle-Van't Hoff relation in the case of penetrating solutes:

$$\sigma_x \pi_x^o + \alpha = \frac{\beta}{V - b} \quad (3.44)$$

If we vary π_x^o and measure V , we can then use Equation 3.44 to obtain the reflection coefficients for various nonelectrolytes in the external solution.

Reflection coefficients of chloroplasts. When the refinements introduced by reflection coefficients are taken into account, we can use osmotic responses of cells and organelles to quantitatively describe the permeability properties

of their membranes. As a specific application of Equation 3.44, the progressive addition of hydroxymethyl groups in a series of polyhydroxy alcohols causes the reflection coefficients to steadily increase from 0.00 to 1.00 for pea chloroplasts (Table 3.2). In this regard, the lipid:water solubility ratio decreases in going from methanol to ethylene glycol to glycerol to erythritol to adonitol, i.e., the partition coefficient K_x decreases. Since the permeability coefficient P_x equals $D_x K_x / \Delta x$ (Eq. 1.9), we expect a similar decrease in P_x as hydroxymethyl groups are added. Figure 3.6 clearly shows that as the permeability coefficient decreases, the reflection coefficient generally increases. Consequently, the increase in σ_x of alcohols as $-\text{CHOH}$ groups

are added can be interpreted as simply a lowering of K_x . (As we go from methanol to adonitol, D_x also decreases, perhaps by a factor of 3, while K_x decreases about a thousandfold, so that changes in K_x are the predominant influence on P_x and σ_x in this case.) The reflection coefficients of six-carbon polyhydroxy alcohols, such as sorbitol and mannitol, are essentially unity for pea chloroplasts. This indication of relative impermeability suggests that these compounds would serve as suitable osmotica in which to suspend chloroplasts, as is indeed the case.

We may reasonably expect partition coefficients for the chloroplast membranes to be lower for amino acids than for adonitol. Since σ_{adonitol} is 1.00 for pea chloroplasts, we would therefore anticipate that the reflection coefficients of amino acids would be near unity. However, Table 3.2 shows

Table 3.2

Reflection coefficients of chloroplasts from *Pisum sativum* for alcohols and amino acids.

The reflection coefficients here apply to the pair of membranes surrounding the organelles, this being the barrier to solute entry or exit encountered in the plant cell. Actual analysis of osmotic responses of pea chloroplasts to various solutes in the external solution, using Equation 3.44, was simplified by noting that sucrose is essentially impermeable ($\sigma_{\text{sucrose}} = 1.00$).

Alcohol	σ_x	Amino acid	σ_x	Amino acid	σ_x
Methanol	0.00	Glycine	0.03	L-threonine	0.02
Ethylene glycol	0.40	L-alanine	0.01	L-serine	0.04
Glycerol	0.63	L-isoleucine	0.33	L-methionine	0.04
Meso-erythritol	0.90	L-valine	0.53		
Adonitol	1.00	L-leucine	0.56		

SOURCES: P. S. Nobel and C.-t. Wang, *Biochimica et Biophysica Acta* **211**: 79-87 (1970) and C.-t. Wang and P. S. Nobel, *Biochimica et Biophysica Acta* **241**: 200-212 (1971). Data reprinted by permission.

that this is not the case, as five of the amino acids have a σ_x of 0.04 or less for pea chloroplasts. L-isoleucine ($\sigma_x = 0.33$), L-valine (0.53), and L-leucine (0.56) have somewhat higher reflection coefficients, but these amino acids all have a branch methyl group on the side chain which apparently hinders their passage across the chloroplast membranes. In contrast, the σ_x s of these same eight amino acids are in all cases near unity for the cellular membranes which have been studied (for example, see Wright and Diamond). The fact that the reflection coefficients of amino acids are low for chloroplasts and high for cells has important physiological implications. Amino acids needed for protein synthesis in chloroplasts can then readily diffuse in from the cytoplasm. Also, amino acids produced as photosynthetic products can move out into the cytoplasm without the utilization of any energy for active transport. On the other hand, if amino acids could readily diffuse across cellular membranes, they would rapidly be lost from the cell.

Since the reflection coefficients of amino acids for pea chloroplasts are much less than predictions based on the partition coefficients, the permeation of amino acids into the chloroplasts may not be governed by the partition of these compounds between membrane lipids and water. Evidently, carriers passively shuttle the amino acids into and out of chloroplasts and thereby lower the effective reflection coefficients, an example of facilitated diffusion mentioned earlier, pp. 136–137 (see Nobel and Cheung). When the concentration of an amino acid is raised above 100 mM in the external solution bathing the chloroplasts, the reflection coefficients increase to values near unity. This is interpreted as a saturation of the carriers used for facilitated diffusion, and can be analyzed using a Michaelis-Menten type of formalism (Eq. 3.27). If an aliphatic amino acid—L-alanine, L-isoleucine, L-valine, and L-leucine—is placed in the external solution with glycine, the chloroplast osmotic responses indicate competition for the same carrier. A second carrier for L-serine, L-threonine, and L-methionine is also demonstrated. In short, three criteria for the use of carriers in facilitated diffusion, viz., specificity, saturation, and competition, can all be demonstrated using the osmotic responses of chloroplasts as interpreted by the modified form of the Boyle-Van't Hoff relation (Eq. 3.44). Hence, the rather esoteric concepts of irreversible thermodynamics can be applied in a relatively simple manner to gain insights into the physiological attributes of membranes.

Problems

3.1 At the beginning of this chapter we calculated that an average concentration of $1 \mu\text{M}$ excess monovalent anions can lead to a -100 mV potential change across

the surface of a spherical cell $30\text{ }\mu\text{m}$ in radius. (a) If the same total amount of charge were all concentrated in a layer 3 nm ($30\text{ }\text{\AA}$) thick at the surface of the sphere, what would be its average concentration there? (b) If 10^7 sulfate ions are added inside the sphere, what is the new potential difference across the surface? (c) Approximately how much electrical work in joules is required to transport the 10^7 sulfate ions across the surface of the cell?

3.2 For purposes of calculation, let us assume that an external solution is 1 mM KCl , and the solution inside a cell is 160 mM KCl at 20° . (a) If K^+ is in equilibrium across the membrane and activity coefficients are unity, what is the electrical potential drop across the membrane? (b) If K^+ is in equilibrium and mean activity coefficients are calculated from Equation 3.3, what would the membrane potential be? (c) If $1\text{ mM K}_3\text{ATP}$ —which fully dissociates to 3K^+ and ATP^{3-} —is added inside the cell and $\text{K}^+-\text{ATP}^{3-}$ can be considered to act as an ion pair, what is $\gamma_{\text{K-ATP}}$ and a_{ATP} ?

3.3 Consider a cell with a membrane potential (E_M) of -118 mV at 25° . Suppose that the external solution contains 1 mM KCl , 0.1 mM NaCl , and 0.1 mM MgCl_2 , while the internal concentration of K^+ is 100 mM , of Ca^{++} is 1 mM , and of Mg^{++} is 10 mM . Assume that activity coefficients are unity. (a) Are K^+ and Mg^{++} in equilibrium across the membrane? (b) If Na^+ and Ca^{++} are in equilibrium, what are their concentrations in the two phases? (c) If Cl^- is 177 mV away from equilibrium, such that the passive driving force on it is outward, what is its Nernst potential and the internal concentration of chloride? (d) What is $J_{\text{Cl}}^{\text{in}}/J_{\text{Cl}}^{\text{out}}$ for passive fluxes? (e) What are $\Delta\mu_{\text{Cl}}$ and $\Delta\mu_{\text{Mg}}$ across the membrane?

3.4 A 10 mM KCl solution at 25° is placed outside a cell formerly bathed in 1 mM KCl . (a) Assuming that some of the original solution adheres to the cell and that the ratio of mobilities ($u_{\text{Cl}}/u_{\text{K}}$) is 1.04 , what diffusion potential would be present? (b) After a sufficient lapse of time, assume that equilibrium is reached in the bathing solution. The membrane may contain many carboxyl groups ($-\text{COOH}$) whose H^+ s will dissociate. The ensuing negative charge will attract K^+ , and its concentration near the membrane may reach 200 mM . What type and how large a potential would be associated with this situation? (c) Suppose that 10 mM NaCl is also in the external solution (with the 10 mM KCl), and that internally there is 100 mM K^+ , 10 mM Na^+ , and 100 mM Cl^- . Assume that $P_{\text{Na}}/P_{\text{K}}$ is 0.20 and $P_{\text{Cl}}/P_{\text{K}}$ is 0.01 . What diffusion potential would be expected across the membrane? (d) What would E_M be if $P_{\text{Cl}}/P_{\text{K}}$ were 0.00 ? If $P_{\text{Na}}/P_{\text{K}}$ and $P_{\text{Cl}}/P_{\text{K}}$ were both 0.00 ? Assume that other conditions are as in (c).

3.5 Consider an illuminated spherical spongy mesophyll cell $40\text{ }\mu\text{m}$ in diameter, containing 50 spherical chloroplasts $4\text{ }\mu\text{m}$ in diameter. (a) Some monovalent anion produced by photosynthesis has a steady state net flux out of the chloroplasts of $1\text{ picomole/cm}^2\text{-sec}$. If this photosynthetic product is not changed in any of the cellular compartments, what is the net passive flux out of the cell in the steady state? (b) If the passive influx of the above substance into the cell at 25° is $0.1\text{ picomole/cm}^2\text{-sec}$, what is the difference in its chemical potential across the

cellular membrane? (c) Suppose that when the cell is placed in the dark, the influx and the efflux both become $0.01 \text{ picomole/cm}^2\text{-sec}$. If the plasmalemma potential is -118 mv (inside negative) and the same concentration occurs on the two sides of the membrane, what can be said about the energetics of the two fluxes? (d) If 1 ATP is required per ion transported, what is the rate of ATP consumption in (c)? Express your answer in $\mu\text{moles/sec}$ per liter of cellular contents.

3.6 The energy of activation for crossing biological membranes can represent the energy required to break hydrogen bonds between certain nonelectrolytes and the solvent water, e.g., to enter the cell the solute must first dissolve in the lipid phase of the membrane and thus the hydrogen bonds with water must be broken. (a) What will be the Q_{10} for the influx of a solute which forms one H-bond per molecule with water if we increase the temperature from 10° to 20° ? (b) How many hydrogen bonds would have to be ruptured per molecule to account for a Q_{10} of 3.3 under the conditions of (a)?

3.7 Suppose that carriers in the plasmalemma can shuttle K^+ and Na^+ into the cell. We will let the Michaelis constant (K_j) be 0.010 mM for the K^+ carrier and 1.0 mM for the Na^+ carrier, while the maximum influx of either ion is $1.0 \text{ picomole/cm}^2\text{-sec}$. (a) What is the relative influx of K^+ to Na^+ when the external concentration of each ion is 0.010 mM ? (b) What is $J_{\text{K}}^{\text{in}}/J_{\text{Na}}^{\text{in}}$ when the external concentration of each ion is 100 mM ? (c) If the entry of K^+ is by facilitated diffusion only, what is the rate of potassium entry when c_{K}^o is 0.1 mM and ATP is being hydrolyzed at the rate of $1 \mu\text{mole/cm}^2\text{-sec}$?

3.8 Consider a cell whose membrane has a hydraulic conductivity coefficient (L_P) of $10^{-4} \text{ cm/sec-bar}$. Initially, no net volume flow occurs when placed in a solution having the following composition; sucrose ($\pi_j^o = 2 \text{ bars}$, $\sigma_j = 1.00$), ethanol (1 bar , 0.30), and glycerol (1 bar , 0.80). The external solution is at atmospheric pressure while P^i is 5 bars . Inside the cell the osmotic pressure caused by glycerol is 2 bars , sucrose and ethanol are initially absent, and other substances having an osmotic pressure of 10 bars are present. (a) What is the mean reflection coefficient for the external solution? (b) What is the mean reflection coefficient for the internal solutes other than glycerol? (c) Suppose that some treatment makes the membrane non-selective for all solutes present. Is there then a net volume flow? (d) If another treatment makes the membrane impermeable to all solutes present, what would be the net volume flow?

3.9 Consider a cell at the point of incipient plasmolysis in an external solution containing 0.3 molal sucrose, an impermeable solute. The concentration of glycine which just causes plasmolysis is 0.4 molal . Assume that no water enters or leaves the cell during the plasmolytic experiments. (a) What is the reflection coefficient of glycine for the cellular membrane? (b) Suppose that chloroplasts isolated from such a cell have the same osmotic responses as in Problem 2.5. What is the volume of such chloroplasts *in vivo*? Assume that activity coefficients are unity and that the temperature is 20° . (c) Suppose that chloroplasts are isolated in 0.3 molal sucrose,

which has a reflection coefficient of 1.00 for the chloroplasts. If 0.1 mole of glycine is then added per liter of isolation medium and the chloroplast volume is $23 \mu\text{m}^3$, what is the reflection coefficient of glycine for the chloroplast membranes? (d) What is the external concentration of glycerol ($\sigma_j = 0.60$) in which the chloroplasts have the same initial volume as in 0.3 M sucrose? What is the chloroplast volume after a long time in the glycerol solution?

References

- Agin, D. P. (ed.). 1972. *Perspectives in Membrane Biophysics: A Tribute to Kenneth S. Cole*, Gordon and Breach, New York.
- Anderson, W. P. 1972. Ion transport in the cells of higher plant tissues. *Annual Review of Plant Physiology* **23**: 51-72.
- Bittar, E. E. 1970. *Membranes and Ion Transport*, Vol. I, Interscience (Wiley), New York.
- Briggs, G. E., A. B. Hope, and R. N. Robertson. 1961. *Electrolytes and Plant Cells*, F. A. Davis, Philadelphia.
- Bull, H. B. 1964. *An Introduction to Physical Biochemistry*, F. A. Davis, Philadelphia.
- Cole, K. S. 1968. *Membranes, Ions, and Impulses*, University of California Press, Berkeley and Los Angeles.
- Dainty, J. 1962. Ion transport and electrical potentials in plant cells. *Annual Review of Plant Physiology* **13**: 379-402.
- Dainty, J. 1963. Water relations of plant cells. *Advances in Botanical Research* **1**: 279-326.
- Dainty, J., and B. Z. Ginzburg. 1964. The reflection coefficient of plant cell membranes for certain solutes. *Biochimica et Biophysica Acta* **79**: 129-137.
- Dainty, J., and A. B. Hope. 1959. Ionic relations of cells of *Chara australis*. *Australian Journal of Biological Sciences* **12**: 395-411.
- Davson, H., and J. F. Danielli. 1952. *The Permeability of Natural Membranes*, 2nd ed., Cambridge University Press, Cambridge.
- De Groot, S. R., and P. Mazur. 1969. *Non-Equilibrium Thermodynamics*, North-Holland, Amsterdam.
- Diamond, J. M., and E. M. Wright. 1969. Biological membranes: the physical basis of ion and nonelectrolyte selectivity. *Annual Review of Physiology* **31**: 581-646.
- Edsall, J. T., and J. Wyman. 1958. *Biophysical Chemistry*, Vol. I, Academic Press, New York.
- Epstein, E. 1972. *Mineral Nutrition of Plants: Principles and Perspectives*, Wiley, New York.
- Goldman, D. E. 1943. Potential, impedance, and rectification in membranes. *Journal of General Physiology* **27**: 37-60.
- Gutknecht, J., and J. Dainty. 1968. Ionic relations of marine algae. *Oceanography and Marine Biology, An Annual Review* **6**: 163-200.

- Higinbotham, N. 1970. Movement of ions and electrogenesis in higher plant cells. *American Zoologist* **10**: 393-403.
- Higinbotham, N., J. S. Graves, and R. F. Davis. 1970. Evidence for an electrogenic ion transport pump in cells of higher plants. *Journal of Membrane Biology* **3**: 210-222.
- Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *Journal of Physiology* **108**: 37-77.
- Hope, A. B. 1971. *Ion Transport and Membranes*, Butterworths, London; University Park Press, Baltimore.
- Katchalsky, A., and P. F. Curran. 1967. *Nonequilibrium Thermodynamics in Biophysics*, Harvard University Press, Cambridge.
- Kedem, O., and A. Katchalsky. 1958. Thermodynamic analysis of the permeability of biological membranes to nonelectrolytes. *Biochimica et Biophysica Acta* **27**: 229-246.
- Lakshminarayanaiah, N. 1969. *Transport Phenomena in Membranes*, Academic Press, New York.
- Läuger, P. 1972. Carrier-mediated ion transport. *Science* **178**: 24-30.
- Lehninger, A. L. 1970. *Biochemistry*, Worth, New York.
- MacInnes, D. A. 1961. *The Principles of Electrochemistry*, rev. 2nd ed., Dover, New York.
- MacRobbie, E. A. C. 1962. Ionic relations of *Nitella translucens*. *Journal of General Physiology* **45**: 861-878.
- MacRobbie, E. A. C. 1970. The active transport of ions in plant cells. *Quarterly Reviews of Biophysics* **3**: 251-295.
- MacRobbie, E. A. C. 1971. Fluxes and compartmentation in plant cells. *Annual Review of Plant Physiology* **22**: 75-96.
- Morris, J. G. 1968. *A Biologist's Physical Chemistry*, Addison-Wesley, Reading (Mass.).
- Neame, K. D., and T. C. Richards. 1972. *Elementary Kinetics of Membrane Carrier Transport*, Wiley, New York.
- Nobel, P. S. 1969. The Boyle-Van't Hoff relation. *Journal of Theoretical Biology* **23**: 375-379.
- Nobel, P. S., and Y.-N.S. Cheung. 1972. Two amino acid carriers in pea chloroplasts. *Nature* **237**: 207-208.
- Prigogine, I. 1967. *Thermodynamics of Irreversible Processes*, 3rd ed., Interscience, New York.
- Raven, J. A. 1969. Action spectra for photosynthesis and light-stimulated ion transport processes in *Hydrodictyon africanum*. *New Phytologist* **68**: 45-62.
- Robinson, R. A., and R. H. Stokes. 1970. *Electrolyte Solutions*, rev. 2nd ed., Butterworths, London.
- Ruch, T. C., and H. D. Patton. 1965. *Physiology and Biophysics*, Saunders, Philadelphia.
- Spanner, D. C. 1964. *Introduction to Thermodynamics*, Academic Press, New York.

- Spanswick, R. M. 1972. Evidence for an electrogenic ion pump in *Nitella translucens*. *Biochimica et Biophysica Acta* **288**: 73-89.
- Stein, W. D. 1967. *The Movement of Molecules Across Cell Membranes*, Academic Press, New York.
- Teorell, T. 1949. Membrane electrophoresis in relation to bio-electrical polarization effects. *Archives des Sciences Physiologiques* **3**: 205-219.
- Ussing, H. H. 1949. The distinction by means of tracers between active transport and diffusion. *Acta Physiologica Scandinavica* **19**: 43-56.
- Vorobiev, V. N. 1967. Potassium ion activity in the cytoplasm and the vacuole of cells of *Chara* and *Griffithsia*. *Nature* **216**: 1325-1327.
- White, A., P. Handler, and E. L. Smith. 1973. *Principles of Biochemistry*, 5th ed., McGraw-Hill, New York.
- Wright, E. M., and J. M. Diamond. 1969. Patterns of non-electrolyte permeability. *Proceedings of the Royal Society, Series B*, **172**: 227-271.
- Yariv, J., I. Z. Steinberg, A. J. Kalb, R. Goldman, and E. Katchalski. 1972. Permease as a rotatory carrier. *Journal of Theoretical Biology* **35**: 459-465.



LIGHT

As we all know, the sun helps maintain temperatures on the earth suitable for plants and animals. The sun is also the ultimate source of the energy needed to sustain life. Through a series of nuclear reactions taking place within the sun, nuclear mass is converted into energy in accordance with Einstein's famous relation, $E = mc^2$. Such conversion of mass to energy enables the sun to maintain an extremely high temperature and to radiate a great amount of energy into space. Some of this radiant energy is incident on the earth, of which only a fraction is absorbed by plants. This absorption initiates the energy flow through the biosphere (plants and animals and that portion of the earth they inhabit). The first step in the utilization of sunlight is the conversion of its radiant energy into various forms of chemical energy by the primary processes of photosynthesis. Such chemical energy may then be stored in the plants, mainly in the form of carbohydrates. Plants act as the direct source of energy for herbivorous animals and indirectly for carnivores which eat the herbivorous animals. Omnivorous animals such as man feed upon plants as well as other animals—again, the ultimate source of energy being the solar radiation trapped by photosynthesis. Without a continuous energy input such as occurs from the sun, plants and animals would drift toward equilibrium and consequent cessation of life.

Sunlight also regulates certain activities of many plants and animals by acting as a trigger. The energy to carry out such activities is supplied by metabolic reactions, not directly by the light itself. Examples where light acts as a trigger are vision, phototaxis, phototropism, and phytochrome regulation of certain plant processes.

In this chapter we will be primarily concerned with the physical nature of light and the mechanism of light absorption by molecules. We will discuss how molecular states so excited can promote endergonic (energy-requiring) reactions or be dissipated by other types of de-excitation processes. In

Chapter 5 we will consider the primary events of photosynthesis and in Chapter 6 the bioenergetics of energy conversion, especially that taking place in organelles. In Chapter 7 we will demonstrate how the net energy input by radiation is dissipated by a leaf.

To help introduce the topic of light, we shall briefly review certain historical developments in the understanding of its nature (see Seliger and McElroy, or Halliday and Resnick for further historical details). In 1666 Newton showed that a prism could disperse white light into many different colors, suggesting that such radiation was a mixture of a large number of components. Soon thereafter, Huygens proposed that the propagation of light through space could be by wave motion, while in the early 1800s Young attributed interference properties to the wave character of light. However, a wave theory of light was not generally accepted until about 1850 when Foucault demonstrated that light travels more slowly in a dense medium such as water than in a rare medium such as air, one of the predictions of the wave theory. In 1887 Hertz discovered that light striking the surface of a metal could cause the release of electrons from the solid. He also found that light above a certain wavelength could not eject any electrons at all, no matter what the total energy in the beam. This important result was contradictory to the then accepted wave theory of light. In an important departure from wave theory, Planck proposed in 1901 that radiation was particle-like, i.e., light was describable as discrete packets, or quanta, each of specific energy. In 1905 Einstein explained the photoelectric effect of Hertz as a special example of the particle nature of light, indicating that the absorption of a light quantum of sufficiently short wavelength by an electron in the metal could supply enough energy to cause the ejection or release of that electron—if the wavelengths were longer, then the individual quanta were not energetic enough to eject any electrons. The intriguing wave-particle duality of light has subsequently become described in a consistent manner through the development of quantum mechanics. (Although this text does not require a background in quantum mechanics, some knowledge of this field is really essential for a comprehensive understanding of light.) Both wave and particle attributes of light are necessary for a complete description of radiation, and we will consider both aspects in this chapter.

Wavelength and energy

Light is often defined as radiation perceived by the human eye. Although such a definition may be strictly correct, the word *light* is frequently used

to refer to a larger class of electromagnetic waves. In this section we will discuss the range of electromagnetic radiation that is important in plant and animal physiology, including the subdivisions into various wavelength intervals. The wavelength of light will be related to its energy. After noting the units for radiant flux and illumination, we will briefly consider some of the characteristics of solar radiation reaching the earth.

Light waves. Our concern here is with the regular and repetitive changes in the intensity of the minute electric and magnetic fields which indicate the passage of a light wave (cf. Fig. 4.1). Light can travel in a solid (glass), in a liquid (water), in a gas (air), and even in a vacuum (between the sun and the earth's atmosphere). One way to characterize light is by its wavelength—the separation in space between successive points of the same phase, such as between two successive peaks of a wave train (Fig. 4.1). A wavelength is the distance per *cycle* of the wave. One unit for wavelengths of light is the Ångström (Å), which equals 10^{-8} cm or 10^{-10} m (App. III). Another is the millimicron. However, the presently preferred unit for wavelengths of

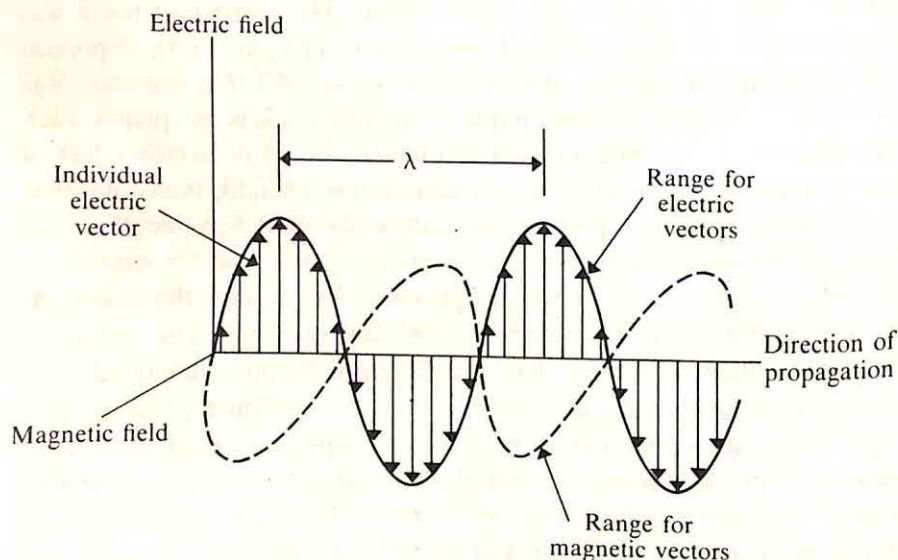


Figure 4.1

Light can be represented by an electromagnetic wave corresponding to oscillations of the local electric and magnetic fields. The oscillating electric vectors at a particular instant in time are indicated by arrows in the diagram. A moment later, the entire pattern of electric and magnetic fields will shift in the direction of propagation of the wave. A wavelength (λ) is the distance between two successive points of the same phase along the wave.

Table 4.1**Definitions and characteristics of the various wavelength regions of light.**

The ranges of wavelengths leading to the sensation of a particular color are somewhat arbitrary and vary with individuals. Both frequencies and energies in the table refer to the particular wavelength indicated in column 3 for each wavelength interval. Wavelength magnitudes are those in a vacuum.

Color	Approximate wavelength range (nm)	Representative wavelength (nm)	Frequency (cycles/sec) (Hertz)	Energy	
				(ev per photon)	(kcal/"mole" of photons)
Ultraviolet	below 400	254	11.80×10^{14}	4.88	112.5
Violet	400-425	410	7.31×10^{14}	3.02	69.7
Blue	425-490	460	6.52×10^{14}	2.70	62.2
Green	490-560	520	5.77×10^{14}	2.39	55.0
Yellow	560-585	580	5.17×10^{14}	2.14	49.3
Orange	585-640	620	4.84×10^{14}	2.00	46.2
Red	640-740	680	4.41×10^{14}	1.82	42.1
Infrared	above 740	1400	2.14×10^{14}	0.88	20.4

light is the nanometer (nm)—like the millimicron, it is 10^{-9} m—and it will be used in this text ($1 \text{ nm} = 10 \text{ \AA}$).

The wavelength regions of major interest in biology are the ultraviolet, the visible, and the infrared (Table 4.1). Wavelengths below about 400 nm are referred to as ultraviolet (UV)—meaning on the other side of, or beyond violet, in the sense of having a shorter wavelength. The visible region extends from approximately 400 nm to 740 nm and is subdivided into various bands such as blue, green, or red (Table 4.1). These divisions are based on the subjective color experienced by humans. The infrared (IR) region has wavelengths longer than those of the red end of the visible spectrum, up to approximately $40 \mu\text{m}$.

Besides wavelength, we can also characterize a light wave by its frequency of oscillation, ν , and by a velocity of propagation that has the magnitude v . These three quantities are related as follows:

$$\lambda\nu = v \quad (4.1)$$

where λ is the wavelength. For all wavelengths the speed of light in a vacuum is a constant, generally designated as c , which experimentally equals $299,792 \pm 1 \text{ km/sec}$, or $3.00 \times 10^8 \text{ m/sec}$ (see Halliday and Resnick, or Monk for measurements of the speed of light). Light passing through a medium other than a vacuum has a speed less than c . For example, light having a wavelength of 589 nm in a vacuum is decreased in speed 0.03%

by air, 25% by water, and 40% by dense flint glass. The wavelength undergoes a decrease in magnitude equal to the decrease in the speed of light in these various media, while the unchanging property of a wave propagating through different media is its frequency. Note that ν is the frequency of the oscillations of the local electric and magnetic fields illustrated in Figure 4.1.

For light, $\lambda_{\text{vacuum}}\nu$ equals v_{vacuum} by Equation 4.1, where v_{vacuum} is the constant c . Therefore, if we know the wavelength in a vacuum, we can calculate the frequency. In fact, the wavelengths given for light generally refer to values in a vacuum, as is the case for columns 2 and 3 in Table 4.1 (λ s in air differ only slightly from the magnitudes listed). As a specific example, let us select a wavelength in the blue region of the spectrum, e.g., 460 nm. By Equation 4.1, the corresponding frequency equals $c/\lambda_{\text{vacuum}}$, or $(3.00 \times 10^8 \text{ m/sec})/(460 \times 10^{-9} \text{ m/cycle})$, which is 6.52×10^{14} cycles/sec for such blue light. Since ν does not change from medium to medium, it is often desirable to describe light by its frequency, as has been done in column 4 of Table 4.1.*

Energy of light. In addition to having wave characteristics, light can also exhibit particle-like properties, as in the case of the photoelectric effect mentioned above. Thus, light can act as if it were divided (or quantized) into discrete units, which we call photons. The light energy (E_λ) carried by a photon is

$$E_\lambda = h\nu = hc/\lambda_{\text{vacuum}} \quad (4.2)$$

where h is a fundamental physical quantity called *Planck's constant*. By Equation 4.2, a photon of light has an energy directly proportional to its frequency, and inversely proportional to its wavelength in a vacuum. For most applications in this book, we will describe light by its wavelength λ . By way of emphasizing that the light energy of a photon depends on its wavelength, we have used the symbol E_λ in Equation 4.2. Finally, a *quantum* (plural: quanta) refers to the light energy carried by a photon, i.e., $h\nu$ represents a quantum of electromagnetic energy. The terms "photons" and "quanta" are sometimes used interchangeably, a usage that is generally clear but strictly speaking not correct.

The introduction of the constant h by Planck in the early 1900s represented a great departure from the accepted wave theory of light. It substan-

* The wavelength of light is generally expressed in nm, not nm/cycle, and ν is considered to have units of sec^{-1} (not cycles/sec) for purposes of calculation.

tially modified the classical equations describing radiation and provided a rational basis for determining the energy of a given number of photons. Since frequency has the units of reciprocal time, Equation 4.2 indicates that Planck's constant dimensionally is energy $\times$ time, the appropriate unit depending on the particular application. For instance, h is equal to 6.626×10^{-27} erg-sec, 0.4136×10^{-14} ev-sec, or 1.584×10^{-37} kcal-sec (App. II).

For many purposes, we may find it convenient to express the energies of individual photons in electron volts (ev). Ionization potentials and the spacings between energy levels in atoms and molecules are often so expressed. Blue light of 460 nm has a frequency of 6.52×10^{14} cycles/sec (Table 4.1). From Equation 4.2, a quantum of such light has an energy $h\nu$ of $(0.4136 \times 10^{-14} \text{ ev-sec})(6.52 \times 10^{14}/\text{sec})$, or 2.70 ev. Using Equation 4.2 and the appropriate numerical value of hc , we can also calculate energies in electron volts from the wavelengths of light. Since hc is equal to 1240 ev-nm (App. III), blue light of 460 nm—strictly speaking, this is the wavelength in a vacuum—has an energy of $(1240 \text{ ev-nm})/(460 \text{ nm})$, or 2.70 ev. Table 4.1, column 5, gives the energy in ev/photon at various wavelengths in the ultraviolet, visible, and infrared.

The customary energy unit for chemical reactions is the kcal/mole, and it is often advantageous to express light energy in a comparable way. The unit analogous to moles but used for radiation is the *Einstein*, which has unit analogous to moles but used for radiation is the *Einstein*, which has Avogadro's number (6.023×10^{23}), N , of photons. Although not strictly correct, a "mole" of photons is often used to refer to 6.023×10^{23} photons.* Using Equation 4.2 and the appropriate numerical value of h , we can calculate that the energy of an Einstein (mole) of photons of blue light of wavelength 460 nm is $Nh\nu$, or $(6.023 \times 10^{23}/\text{mole})(1.584 \times 10^{-37} \text{ kcal-sec})(6.52 \times 10^{14}/\text{sec})$, which equals 62.2 kcal/mole. Likewise, since Nhc equals 28,600 kcal-nm/mole (App. III), we can also calculate the energy of 460 nm photons using their vacuum wavelength, which gives $(28,600 \text{ kcal-nm/mole})/(460 \text{ nm})$, or 62.2 kcal/mole. The energies of various wavelengths of light are presented in kcal/mole of photons in the last column of Table 4.1.

We can see that quanta of visible light represent relatively large amounts of energy. The hydrolysis of ATP, the main currency for chemical energy

* The term *mole* refers of course to a mass equal to the molecular weight in grams, a unit which contains N molecules (App. IV). Since light has no mass, mole is not really an appropriate expression, although such usage is widespread and the actual meaning is clear.

in biology, yields about 10 to 14 kcal/mole under physiological conditions (see Ch. 6), while blue light has about five times as much energy/mole of photons. Quanta of ultraviolet light represent even higher energies than those of visible light, e.g., 254 nm photons have 113 kcal of radiant energy/mole (Table 4.1). This is greater than the carbon-carbon bond energy of 83 kcal/mole or the oxygen-hydrogen bond energy of 111 kcal/mole. The high quantum energy of UV radiation underlies its mutagenic and bactericidal action, since it is energetic enough to cause disruption of certain covalent bonds.

The photoelectric effect, where light leads to the ejection of electrons from the surface of a metal, clearly illustrates the distinction between light energy and the energy of its quanta. The amount of energy required to remove electrons from the surface of metallic sodium was found by R. A. Millikan to be 1.82 eV/electron, or 41.9 kcal/mole of electrons, which corresponds to light of wavelength 683 nm (see Halliday and Resnick).^{*} Quanta of light energy representing wavelengths of 683 nm or less thus can lead to a photoelectric effect for sodium. However, no matter how much light energy for wavelengths beyond 683 nm is absorbed, electrons are not ejected from the surface. Hence, the energy of quanta specifies the quality of the radiation, while the total light energy is a measure of its quantity without indicating how many photons are actually involved.

Absorption of radiation by an atom or molecule leads to a more energetic state of the absorbing species. Such energetic states can also be produced by collisions resulting from the random thermal motion of the molecules. The higher the temperature, the greater is the average kinetic energy of the atoms and molecules, and the greater is the probability of achieving a relatively energetic state by collision. The number of molecules having a particular kinetic energy can often be approximated by the Boltzmann energy distribution (see p. 121). By Equation 3.21 ($n(E) = n_{\text{total}} e^{-E/kT}$), the fraction of atoms or molecules having a kinetic energy of molecular Brownian motion of E or greater at thermal equilibrium equals the Boltzmann factor, $e^{-E/kT}$, where k is the Boltzmann constant (8.617×10^{-5} eV/molecule-°K, App. II). Therefore, the number of molecules with kinetic energy in excess of E at a given temperature exponentially decreases with increasing values of E . Energies of molecules are generally of the order of magnitude of kT , which equals (8.617×10^{-5} eV/molecule-°K)(298°K), or 0.026 eV/molecule, at a temperature of 25° (298°K). For instance, the average kinetic

^{*} For his careful work on the photoelectric effect, Millikan was awarded the Nobel prize in physics in 1923.

energy of translation for molecules in a gas is $(3/2)kT$, which equals 0.039 ev at 25°.

Next, we will use the Boltzmann energy distribution to estimate the fraction of the molecules that might be expected to have a certain minimum kinetic energy, namely, 0.1 ev at 25°. For an E of 0.1 ev at 25°, the Boltzmann factor, $e^{-E/kT}$, equals $e^{-(0.1 \text{ ev})/(0.026 \text{ ev})}$, or $e^{-3.8}$, which is 0.022 ($kT = 0.026$ ev at 25°). Therefore, at 25° only about 2% of the molecules would have a kinetic energy of at least 0.1 ev resulting from thermal collisions. On the other hand, the absorption of infrared radiation could lead to an excited state of the same energy. Since the energy E for radiation equals $hc/\lambda_{\text{vacuum}}$ (Eq. 4.2), where hc is 1240 ev-nm (App. III), 0.1 ev corresponds to a photon with a wavelength of $(1240)/(0.1)$, or 12,400 nm (12.4 μm), which is in the IR. Absorption of such infrared radiation can increase the rate of certain reactions because molecules having a higher energy are usually more reactive. Turning the argument involving the Boltzmann factor around, at what temperature would a considerable fraction (e.g., $1/e$) of the molecules have a kinetic energy of 0.1 ev or more? In this case, $e^{-E/kT}$ would then be e^{-1} , where E is 0.1 ev; therefore, T would equal E/k , or $(0.1 \text{ ev/molecule})/(8.617 \times 10^{-5} \text{ ev/molecule-}^\circ\text{K})$, which is 1160°K (887°). In other words, a very high temperature would be required to populate the more energetic states by thermal collisions, a result that could also be achieved by absorbing infrared radiation 12.4 μm in wavelength.

As an example of an excitation energy corresponding to visible light, let us consider a kinetic energy E of 2.70 ev/molecule. In this case, $e^{-E/kT}$ is $e^{-(2.70)/(0.026)}$, or only 4×10^{-46} at room temperature! The chance of a molecule gaining 2.70 ev or more of energy by means of thermal collisions is extraordinarily small. From Table 4.1, 2.70 ev corresponds to blue light of 460 nm. The absorption of such visible light leads to energetic states that otherwise would simply not occur at temperatures encountered by plants and animals. Absorption of the relatively high quantum energy of light promotes the attainment of very improbable energetic states—this point is a key feature in the understanding of photobiology.

It will no doubt be noticed that we are using two different sets of expressions: (1) molecule, mass of molecule, photon, electronic charge, k , kT and (2) mole, molecular weight, Einstein, Faraday, R , RT . A quantity in the second set is simply Avogadro's number N (6.023×10^{23}) times the analogous expression in the first set. The choice between sets is based solely on convenience, and either one is self-consistent. Our examples in the previous paragraphs could equally well have been presented using kcal/mole instead of ev/molecule (1 ev/molecule = 23.06 kcal/mole, App. III), in which case we must replace kT by RT .

Radiant flux and illumination. For many purposes in studying plants, we need to know the total amount of incident light. There are two common ways of measuring such fluxes: the *photometric*, based on the available illuminating power and related as such to the wavelength sensitivity of the human eye, and the *radiometric*, based on the total energy of the radiation.*

By definition, photometric devices do not appreciably respond to radiation in the infrared or the ultraviolet. They are "light" meters in the sense applied to human vision, i.e., they respond to photons in the visible region, in the same way as the light meter on a camera. They often read directly in footcandles or lux. The candle is a unit of luminous intensity, originally based on a standard candle or lamp. The present international unit is called a *candela* (often still referred to as a "candle"), which is $\frac{1}{60}$ of the total light intensity emitted from 1 cm^2 of a blackbody radiator (one that radiates maximally) at the melting temperature of pure platinum (2042°K). The total light emitted in all directions by a source of 1 candela is 4π lumens. One footcandle is simply 1 lumen/ft^2 , and one lux is 1 lumen/m^2 (hence, the lux is a metric unit). To convert footcandles to lux, we simply multiply the number of footcandles by the square feet per square meter, 10.76. Measurement in lux is adequate for certain purposes, particularly where human vision is involved. Since chlorophyll absorbs in the visible region (see Fig. 5.2), photometric units are often employed to describe the illuminations used for photosynthesis. However, such a description can be misleading if the light source or its wavelength distribution is not indicated. For instance, at the same illumination measured in lux, a fluorescent lamp produces about three times as much blue light as a tungsten lamp.

Radiometric devices, e.g., blackened thermocouples, thermopiles, or thermistors, respond to radiant energy and so are sensitive to radiation in the ultraviolet and infrared as well as in the visible. Readings are expressed in energy per unit area and time, such as $\text{cal/cm}^2\text{-min}$, $\text{ergs/cm}^2\text{-sec}$, or $\mu\text{watts/cm}^2$. Calories have widespread use as an energy unit in describing heating or cooling of aqueous solutions as well as for discussing the energetics of biochemical reactions, while ergs and watts are much more in keeping with the sets of units used in the physical sciences. Conversion factors for radiometric units are specifically included in Table 7.2 (also see App. III). If the radiant flux at a specified wavelength is measured in radiometric units, the value can be converted to a photon flux (photons/

* See Bickford and Dunn; Kaufman; and Seliger and McElroy for a discussion of the instruments for photometric and radiometric measurements.

cm²-sec) by using the energy carried by an individual photon as given by Equation 4.2. In general, radiometric units cannot be converted unambiguously to photometric units. In this text *illumination* will refer to a luminous flux that can be measured in lux, while *radiant flux*, or *irradiance* will denote electromagnetic energy per unit area and time. (As in the other chapters, we are using flux to designate the amount of something per unit area and time; many books on light refer to this quantity as the "flux density.")

In choosing the type of lamp for a controlled-environment room for growing plants, we might wish to make *both* radiometric and photometric measurements. For instance, we could estimate the illumination needed for photosynthesis in lux, while other considerations, such as maintaining a reasonable room temperature, would suggest determining total radiant energy. Tungsten lamps are actually comparatively poor sources of visible light, since about 90% of their radiant energy is emitted in the infrared—the actual amount depends on the operating temperature of the filaments—creating appreciable cooling problems when large numbers of such lamps are used. Since typical fluorescent lamps emit only about 10% of their energy in the IR, cooling problems are less severe than with tungsten lamps. On the other hand, the wavelength distribution of the sun's photons in the visible region (Fig. 4.2) is matched far better by tungsten lamps than by fluorescent ones (see Bickford and Dunn; Kaufman; or Seliger and McElroy).

Sunlight. Essentially all energy for life originates in the form of electromagnetic radiation from the sun. Therefore, we will consider some of the characteristics of sunlight incident on the earth, which will also allow us to illustrate the units used for radiation. In radiometric units the flux of solar radiation incident on the earth's atmosphere—the so-called solar constant—is about 2.00 cal/cm²-min.* In Chapter 6 we will consider the solar constant both in terms of the temperatures of radiating bodies and the annual photosynthetic yield, and in Chapter 7 in terms of the energy balance of a leaf. The total daily amount of radiant energy from the sun reaching the earth's surface can be about 700 cal/cm² on a cloudless day in summer—the actual amount varies with latitude, altitude, and atmospheric conditions (see van Wijk). In photometric units sunlight can reach 100,000 lux on the surface of the earth on a cloudless day, leading

* Most recent measurements are in the range 1.98 ± 0.02 cal/cm²-min for the average value of the solar constant (see Munn or van Wijk). The value at any one time varies from the average by $\pm 3\%$ because of the ellipticity of the earth's orbit about the sun; there are additional variations based on changes in solar activity, such as occur for sun spots.

to approximately $0.6 \text{ cal/cm}^2\text{-min}$ in the visible region. If we represent sunlight by yellow light of 580 nm , which has 49.3 kcal of energy/mole by Table 4.1, then the maximum flux of photons in the visible region from the sun is about $(0.6 \text{ cal/cm}^2\text{-min})(1 \text{ min}/60 \text{ sec})/[(49,300 \text{ cal/mole})(1 \text{ mole}/6.023 \times 10^{23} \text{ photons})]$, or $1.2 \times 10^{17} \text{ photons/cm}^2\text{-sec}$ at the earth's surface (see the discussions by Anderson; Seliger and McElroy; and van Wijk).

Figure 4.2 shows the relative number of the sun's photons which impinge on the earth's atmosphere and reach its surface as a function of wavelength. About 5% of the photons incident on the earth's atmosphere are in the ultraviolet (below 400 nm), 28% in the visible, and 67% in the infrared (beyond 740 nm). Most of the ultraviolet fraction of sunlight incident on the atmosphere is prevented from reaching the surface of the earth by ozone

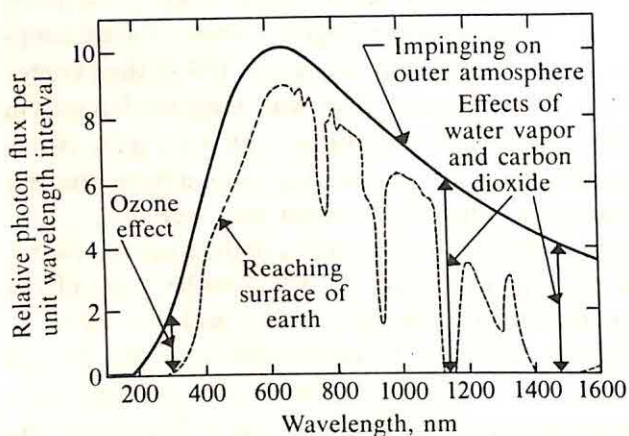


Figure 4.2

Wavelength distributions of the sun's photons incident on the earth's atmosphere and its surface. The curve for the solar radiation on the atmosphere is an idealized one based on Planck's radiation distribution formula. The spectral distribution and amount of solar radiation reaching the earth's surface depends on clouds, other atmospheric conditions, altitude, and the sun's angle in the sky. The pattern indicated by the lower curve is appropriate at sea level on a clear day with the sun nearly overhead. (For further details see Bickford and Dunn; Moon; Seliger and McElroy; and van Wijk.)

(O₃) present in the stratosphere, about 30 km above the earth's surface.* Ozone absorbs some visible radiation (especially near 600 nm), and effectively screens out the shorter ultraviolet rays by absorbing strongly below 300 nm. Much of the infrared from the sun is absorbed by atmospheric water vapor and CO₂ (see Fig. 4.2). Water absorbs strongly near 900 nm and 1100 nm and above 1200 nm, having a major infrared absorption band at 1400 nm (1.4 μ m). Although the amount of water vapor in the air varies with latitude and season, the mean vapor concentration is equivalent to a path of liquid water approximately 2.6 cm thick (see Seliger and McElroy). Because of the substantial absorption of UV and IR by atmospheric gases, the solar radiation at the earth's surface has a larger fraction in the visible region than that incident on the outer atmosphere. In the example in Figure 4.2, about 2% of the photons at the earth's surface are in the ultraviolet, 45% in the visible, and 53% in the infrared.

We can readily appreciate that the wavelength distribution of photons reaching the earth's surface profoundly influences life. For example, the strong absorption of ultraviolet by ozone reduces the potential hazard of mutagenic effects caused by the short wavelength radiation. On the other hand, prior to the advent of appreciable ozone in the upper atmosphere, UV radiation from the sun would be a potent factor in genetic processes. The peak near 680 nm for photons reaching the earth's surface (Fig. 4.2) coincides with the red absorption band of chlorophyll. Vision also utilizes the wavelength region where most of the sunlight reaches the earth. It seems reasonable to suppose that there was evolutionary pressure for selecting photochemical systems able to use light from about 400 to 900 nm.

The shape of the curve depicting the wavelength distribution of photons incident upon the earth's atmosphere can be closely predicted using *Planck's radiation distribution formula*. This expression indicates that the relative photon flux per unit wavelength interval is proportional to $\lambda^{-4}/(e^{hc/\lambda kT} - 1)$, where T is the temperature of the radiation source (see Clayton 1970). Such a formula applies exactly to a perfectly efficient emitter, a so-called *blackbody*. A blackbody is a convenient idealization describing an object which does not absorb or emit only at particular wavelengths. Rather, it absorbs *all* wavelengths—it is uniformly “black” at all wavelengths—and emits in accordance with Planck's radiation distribution formula. This formula is a good approximation for describing radiation from the sun— T is the surface temperature (about 5800°K)—and so it was used to obtain the upper curve in Figure 4.2. For the basic photometric source, the candela, Planck's formula is appropriate with T equal to 2042°K (see p. 170). Also, the

* The absorption of sunlight by this O₃ leads to a pronounced heating of the upper atmosphere.

radiation from a tungsten lamp of a few hundred watts can be fairly well matched by Planck's radiation distribution formula, using a T of 2900°K , i.e., the curve for the relative photon flux from a tungsten lamp has the same shape as the solid line in Figure 4.2, although it is shifted toward longer wavelengths, since the temperature of a tungsten filament is less than that of the sun's surface (see Bickford and Dunn; Kaufman; or Seliger and McElroy).

If we know the temperature of a blackbody, we can actually predict at what wavelength the radiation from it will be at a maximum. To derive such an expression, we differentiate Planck's radiation distribution formula with respect to wavelength and set the derivative equal to zero (see Clayton 1970; also, in App. VI, we discuss the minima and maxima of curves). The relation thus obtained is known as *Wien's displacement law*, which can be stated as follows: the temperature of the source (T) times the wavelength position for maximum *photon* flux (λ_{max}) equals $3.6 \times 10^6 \text{ nm} \cdot ^{\circ}\text{K}$. Since the surface of the sun is at about 5800°K , we expect its λ_{max} to be near $(3.6 \times 10^6 \text{ nm} \cdot ^{\circ}\text{K}) / (5800^{\circ}\text{K})$, or 620 nm, as Figure 4.2 indicates. For a tungsten lamp operating at a temperature of 2900°K (half that of the sun's surface), the position for maximum photon flux shifts to 1240 nm (a doubling of λ_{max} compared with the sun). This is consistent with the fact that tungsten lamps emit primarily infrared radiation. Finally, Planck's radiation distribution formula indicates that any object with a temperature greater than 0°K will emit electromagnetic radiation. From Wien's displacement law, the λ_{max} for a maximum photon flux from a body at 298°K would occur at $(3.6 \times 10^6) / (298)$, or $1.2 \times 10^4 \text{ nm}$, which is $12 \mu\text{m}$, i.e., far into the IR. Plants emit such infrared radiation, and this is a crucial aspect of their overall energy balance, as we will consider in Chapters 6, 7, and 8.

When considering the number of photons available for absorption by pigment molecules, as is relevant for discussing photosynthesis, we generally find it most convenient to use the spectral distribution of *photons* per unit wavelength interval (cf. Fig. 4.2). On the other hand, for applications such as describing the energy gain by leaves exposed to sunlight, we are usually more interested in the spectral distribution of *energy* per unit wavelength interval. To recast Figure 4.2 on an energy basis, we need to divide each point on the curves by its wavelength—the ordinate then becomes "Relative energy flux per unit wavelength interval". Planck's radiation distribution formula also indicates that the relative *energy* flux per unit wavelength interval is proportional to $\lambda^{-5}(e^{hc/\lambda kT} - 1)$. Finally, Wien's displacement law becomes λ_{max} for maximum *energy* output times T equals $2.9 \times 10^6 \text{ nm} \cdot ^{\circ}\text{K}$ (see Clayton 1970, or Halliday and Resnick for further details).

Absorption of light by molecules

In our discussion of light utilization we will first consider the absorption process itself. Only light that is actually absorbed can be effective in producing a chemical change, a principle embodied in the Grotthus-Draper law of photochemistry. This is true whether radiant energy is to be converted to some other form and then stored or simply used as a trigger. Another important principle of photochemistry is the Stark-Einstein law, which specifies that each absorbed photon activates but one molecule. Einstein further postulated that all the energy of the light quantum is transferred to a single electron during the absorption event, resulting in the movement of this electron to a higher energy state. To understand light absorption, we must therefore first consider some of the properties of electrons. We will discuss the fate of the excited electron in the next section. (See Calvert and Pitts; Clayton 1970; Depuy and Chapman; or Turro for a more thorough treatment of the topics in this section.)

Role of electrons in absorption event. From a classical viewpoint, an electron is a charged particle that moves in some orbit around an atomic nucleus. We can readily appreciate that the energy of the electron would depend both on the location of the orbit in space and on how fast the electron moves in its orbit. Thus, the increase in energy of an electron upon absorbing a photon could be used either to transfer that electron into an orbit which lies at a higher energy than the original orbit or to cause the electron to move more rapidly about the nucleus than it did before the light arrived. The locations of various possible electronic orbits and the speeds for electrons moving in them are both limited to certain discrete or "allowed" values, a phenomenon which has been interpreted by quantum mechanics. This means that the energy of an electron in an atom or molecule can change only by certain specific values. Consequently, the total electronic energy of an atom or molecule occurs in discrete amounts. Only light of certain wavelengths will have the proper quantum energy to cause the electron to move from one possible energetic state to another. For light absorption to occur, the energy of a photon as given by Equation 4.2 must equal the difference in energy between some allowed excited state of the atom or molecule and the initial state, the latter usually being the ground (lowest) energy state.

During light absorption an interaction occurs between the electromagnetic field of the light and some electron. Because electrons are charged particles, they will experience a force when they are in an electric field. But light consists of periodic variations in both the local electric and magnetic fields

in the medium through which it is passing (cf. Fig. 4.1). The oscillating electric field of light thus represents a periodic driving force acting on the electrons in matter. This electric field—a vector quantity which has a certain direction in space, such as along the y -axis in Figure 4.1—causes or induces electrons to move. If the frequency of the electromagnetic radiation is such as to cause a large sympathetic oscillation or beating of some electron, that electron is said to be in *resonance* with the light wave. Such a resonating electron corresponds to a so-called *electric dipole* in the molecule, as the electron is forced to move first in a certain direction and then in the opposite one in response to the oscillating electric field of the light. (An electric dipole is simply the local separation of positive and negative charge.) The displacement of the electron back and forth requires energy—in fact, it may take the entire energy of the photon, in which case the quantum is captured or absorbed. The direction and magnitude of the induced electric dipole will depend on the resisting, or restoring forces on the electron provided by the rest of the molecule. These restoring forces depend on the other electrons and the atomic nuclei in the molecule; consequently, they are not the same in different types of molecules. The actual electric dipoles which can be induced in a particular molecule are characteristic of that molecule, which helps explain why each molecular species has its own unique absorption spectrum.

The probability for the absorption of light depends on both its wavelength and the relative orientation of its electromagnetic field with respect to the possible induced oscillations of the electrons in the molecules. Absorption of photons without ejection of electrons from the absorbing species can take place only if the following two conditions exist: (1) the light has the proper energy to get to a discrete excited state of the molecule, i.e., a specific wavelength (cf. Eq. 4.2), and (2) the electric field vector associated with the photon (Fig. 4.1) has a component parallel to the direction of some potential electric dipole in the molecule so that an electron can be induced to oscillate. In other words, the electric field of light must be in the correct direction so that it is able to exert a force on some electron. It must exert this force in the direction of a potential electric dipole so that the electron can be induced to move and thus be able to accept the energy of the photon. The probability for absorption is actually proportional to the square of the cosine of the angle between the electric field vector of light and the direction of the induced electric dipole in the molecule. Within these limits set by the wavelength and the orientation, light energy can be captured by the molecule, placing it in an excited state.

Electron spin and state multiplicity. Light absorption is affected by the

arrangement of electrons in an atom or molecule, which depends among other things on a property of the individual electrons known as their "spin." We can consider that each electron is a charged particle spinning about an axis in much the same way as the earth spins about its axis. Such rotation has an angular momentum or *spin* associated with it. The magnitude of the spin of all electrons is the same; but since spin is a vector quantity, it can have different directions in space. For an electron, only two orientations are actually found, viz., the spin of the electron can be aligned either parallel or antiparallel to the local magnetic field. Even in the absence of an externally applied magnetic field, such as that of the earth or some electromagnet, there is a local internal magnetic field provided by both the moving charges in the nucleus and the motion of the electrons. There is therefore always a magnetic field with which the electron spin can be aligned.

It has proved convenient to express the spin of electrons in units of $h/2\pi$, where h is Planck's constant. In these units, the projection along the magnetic field of the spin for a single electron is either $+\frac{1}{2}$ (e.g., when it is parallel to the local magnetic field), or $-\frac{1}{2}$ (when the spin is in the opposite direction). The total spin of an atom or molecule is the vector sum of the spins of all the electrons, each individual electron having a spin of either $+\frac{1}{2}$ or $-\frac{1}{2}$. The magnitude of this total spin is given the symbol S , an extremely important quantity in spectroscopy.

In order to talk about the spectroscopic properties of various molecules, we will find it convenient to introduce a new term known as the *spin multiplicity*. The spin multiplicity of an electronic state is defined as $2S + 1$, where S is the magnitude of the net spin for the whole atom or molecule. For example, if S equals zero—which means that the spin projections of all the electrons taken along the magnetic field cancel each other—then $2S + 1$ is one, and the state is called a *singlet*. On the other hand, if S equals one, the state is a *triplet* ($2S + 1$ then equals 3). Singlets and triplets are the two most important spin multiplicities encountered in biological systems, and we will consider both in further detail below. When referring to an absorbing species, the spin multiplicity is generally indicated by S for singlet and T for triplet, as we will do here. (When S equals $\frac{1}{2}$ as would occur if there were one unpaired electron in a molecule, $2S + 1$ is 2; such "doublets" occur for free radicals.)

Electrons are found of course only in certain "allowed" regions of space; the particular locus in which some electron can move is referred to as its *orbital*. In the 1920s W. Pauli observed that when an electron is in a given atomic orbital, a second electron having its spin in the same direction is excluded from that orbital. This led to the enunciation of the *Pauli exclusion*

principle of quantum mechanics: when two electrons are in the same orbital, their spins must be in opposite directions. When a molecule has all of its electrons paired in orbitals with their spins in opposite directions, the total spin of the molecule is zero ($S = 0$), and the molecule is in a singlet state ($2S + 1 = 1$), cf. Figure 4.3a. The ground, or unexcited state of most molecules is such a singlet, i.e., all the electrons are in pairs in the lower energy orbitals. When some electron is excited to an unoccupied orbital, two spin possibilities exist. The spins of the two electrons (which are now in different orbitals) may be in opposite directions, as they were when paired in the ground state (Fig. 4.3b). This electronic configuration is still a singlet state. On the other hand, the two electrons may have their spins in the same direction, a triplet state (Fig. 4.3c). (Since the electrons are in different orbitals, their spins can be in the same direction without violating the Pauli principle.) An important rule—first enunciated by Hund, based on empirical observations, and later explained using quantum mechanics—is that the level with the greatest spin multiplicity has the lowest energy. Thus, an excited triplet state is lower in energy than the corresponding excited singlet state, as illustrated in Figure 4.3c; we will consider this phenomenon for chlorophyll.

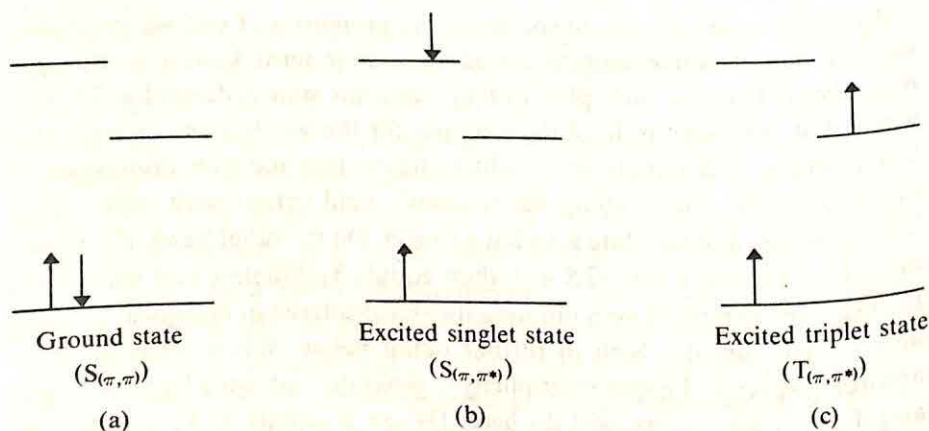


Figure 4.3

Effect of light on a pair of electrons in a molecular orbital. The arrows indicate the directions of the electron spins with respect to the local magnetic field. (a) In the ground state, the two electrons in a filled orbital have their spins in opposite directions. (b) The absorption of a photon can cause the molecule to go to an excited *singlet* state where the spins of the electrons are still in opposite directions. (c) In an excited *triplet* state, the spins of the two electrons are in the same direction.

Molecular orbitals. For a discussion of the light absorption event involving the interaction of an electromagnetic wave with some electron, it is easiest to visualize the electron as a small point located at some specific position in the atom or molecule. For a classical description of the energy of an electron, we can imagine the electron moving in some fixed trajectory or orbit about the nucleus. However, to describe the role of electrons in binding together atoms to form a molecule, it is most convenient to imagine the electron spatially distributed like a cloud of negative charge surrounding the nuclei of adjacent atoms in the molecule. In this last description, involving probability considerations introduced by quantum mechanics, we say that the electrons are located in *molecular orbitals*.

In describing the electronic states of molecules we need to use the language of molecular orbitals in order to discuss adequately the absorption of light by chlorophylls, carotenoids, and other pigments in plants. We must therefore introduce many new terms and, unfortunately, many new concepts which can truly be mastered only by a study of quantum mechanics. Our common experience yields relations such as Newton's laws of motion, which describe events on a scale much larger than atomic or molecular. By contrast, the main application of quantum mechanics is to molecular, atomic, and subatomic dimensions. On such a scale our intuition often fails us. Moreover, many things which otherwise seem to be absolute, such as the position of an object, are really describable only as a probability. We cannot say that an electron is located at such and such a place. Instead, we must be satisfied with knowing only the *probability* of finding an electron in the region of space about some nucleus.

A logical way to begin our discussion of molecular orbitals is to consider the probabilities of finding electrons in given regions about a single atomic nucleus. The region of space in which an electron moves is of course its orbital. The simplest case is the hydrogen atom which contains a single electron moving about a nucleus. If we were to determine the probability of finding that electron in the various regions of space about its nucleus, we would find that it spends most of its time fairly close (within a few Ångströms) of the nucleus, and that the probability distribution is *spherically symmetric* in space. In other words, the chance of finding the electron is the same in all directions about the nucleus. In atomic theory, this spherically symmetric distribution of the electron about the hydrogen nucleus is called an *s* orbital, and the electron is referred to as an *s* electron (see Pauling, or other elementary chemistry texts).

The next simplest case is the helium atom. Helium has two *s* electrons moving in the *s* orbital about its nucleus. By the Pauli exclusion principle

these two electrons must have their spins in opposite directions, as is indeed the case. What happens for lithium, which contains three electrons? Two of its electrons are in the same type orbital as that of helium. This orbital is known as the *K* shell, and represents the spherically symmetrical orbital closest to the nucleus. The third electron is excluded from the *K* shell but occurs in another orbital with spherical symmetry whose probability description indicates that on the average its electrons are further away from the nucleus than is the case for the *K* shell. Electrons in this new orbital are still *s* electrons, but they are now referred to as being in the *L* shell. Beryllium, which has an atomic number of four, can have two *s* electrons in its *K* shell and two more in its *L* shell. What happens when we have 5 electrons moving about a nucleus, as in an uncharged boron atom? It turns out that the fifth electron is in an orbital that is not spherically symmetric about the nucleus. Instead, the probability distribution is in the shape of a dumbbell, although it is still centered about the nucleus (similar in appearance to the distribution depicted in Fig. 4.4b). This new orbital is referred to as a *p* orbital and electrons in it are called *p* electrons. The probability of finding a *p* electron in various regions of space is greatest along some axis through the nucleus and vanishingly small at the nucleus itself.

How do all these various atomic orbitals relate to the spatial distribution of electrons in molecules? A molecule contains more than one atom (except for "molecules" like helium or neon), and certain of the electrons can move between the atoms. Such interatomic motion is of course crucial for holding the molecule together. Fortunately, it still proves possible to describe the spatial localization of electrons in molecules by using suitable linear combinations of the spatial distributions represented by various atomic orbitals centered about the nuclei involved. In fact, molecular orbital theory is concerned with giving the correct quantum-, or wave-mechanical description of the probability of finding electrons in various regions of space in *molecules* by using the already known descriptions for the probability of finding electrons in various locations making up their *atomic* orbitals.

Some of the electrons in molecules are localized about a single nucleus, while others are *delocalized*, or shared between nuclei. For the delocalized electrons, the combination of the various atomic orbitals used to describe the spatial positions of the electrons in three dimensions is consistent with a sharing of the electrons between adjacent nuclei. In other words, the molecular orbitals of the delocalized electrons spatially overlap more than one nucleus. This sharing of electrons is responsible for the chemical bonds that prevent the molecule from separating into its constituent atoms, i.e., the negative electrons moving between the positive nuclei hold the molecule

together by attracting the nuclei of different atoms. Moreover, these delocalized electrons are the ones usually involved in light absorption by molecules.

The lowest energy molecular orbital is a σ orbital, which is analogous in symmetry properties to the s atomic orbital. Nonbonding, or lone-pair electrons contributed by atoms like oxygen or nitrogen occur in n orbitals and retain their atomic character in the molecule. These n electrons are essentially physically separate from the other electrons in the molecule and do not take part in the bonding between nuclei. We shall devote most of our attention to electrons in π molecular orbitals, which are the molecular equivalent of p electrons in atoms. Such π electrons are delocalized in a bond joining two or more atoms, and they are of prime importance in light absorption. In fact, most photochemical reactions and spectroscopic properties of biological importance result from the absorption of photons by the π electrons.

The excitation of a π electron by light absorption can lead to an excited state of the molecule in which the electron moves into a π^* orbital, the asterisk referring to an excited, or high-energy molecular orbital. The probability distributions for electrons in both π and π^* orbitals are illustrated in Figure 4.4 (the circumscribed regions indicate where the electrons are most likely to be found). Figure 4.4a shows a π orbital delocalized between two nuclei; the same clouds of negative charge electrostatically attract both nuclei. Such sharing of electrons in the π orbital helps join the atoms together, hence we refer to this type of molecular orbital as *bonding*. As shown in Figure 4.4b, electrons in a π^* orbital do not help join atoms

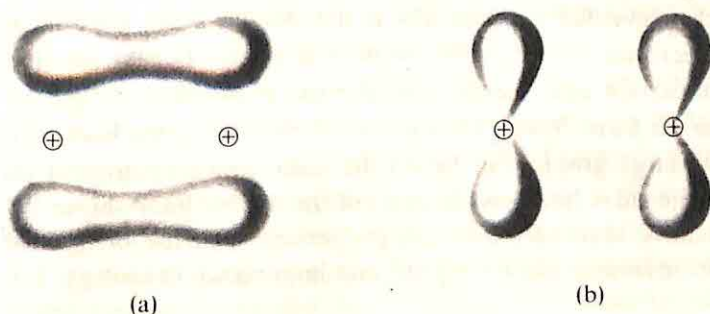


Figure 4.4

Typical π and π^* orbitals, indicating the spatial distribution about the nuclei ($\oplus$) where the greatest probability of finding the electrons occurs: (a) π orbital (bonding) (b) π^* orbital (antibonding).

together, rather, they tend to decrease bonding between atoms in the molecule, since the clouds of negative charge in adjacent atoms are located in a position to repel each other. A π^* orbital is therefore referred to as *antibonding*. The decrease in bonding in going from a π to a π^* orbital results in a less stable (higher energy) electronic state for the molecule, so that a π^* orbital is at a higher energy than a π orbital.

In photochemistry, the energy required to move an electron from the attractive (bonding) π orbital to the antibonding π^* orbital is obtained by the absorption of a photon. For molecules such as chlorophylls and carotenoids, which we will consider later, the π^* orbitals are often only a few electron volts higher in energy than the corresponding π orbitals. In such molecules, the absorption of visible light corresponding to photons with energies from 1.7 to 3.1 eV (Table 4.1) can lead to the excitation of π electrons into the π^* orbitals.

Light energy can cause molecular changes known as *photoisomerizations*. The three main types of photoisomerization are (1) *cis-trans* isomerization about a double bond, (2) a double bond shift, like that implicated in the interconversion between the two forms of phytochrome that we will present below, and (3) a molecular rearrangement involving changes in carbon-carbon bonds, e.g., ring cleavage or formation. *Cis-trans* photoisomerization—which illustrates the consequences of the different spatial distribution of electrons in π and π^* orbitals—makes possible the generally restricted rotation about a double bond.* A double bond has two π electrons; light absorption leads to the excitation of one of them to a π^* orbital. The attraction between the two carbon atoms caused by the remaining π electron is mostly canceled by the antibonding, or repulsive contribution from this π^* electron (cf. Fig. 4.4). Hence, the molecular orbitals of the original two π electrons which prevented rotation about the double bond have been replaced by an electronic configuration permitting relatively easy rotation about the carbon-carbon axis. Let us consider the absorption of light by a molecule in the *cis* form. When the excited π^* electron drops back to a π orbital, the two large groups can be on the same side (the original *cis* form) or on opposite sides (the *trans* isomer) of the double bond. Since the two isomers can have markedly different properties, such use of light to trigger their interconversion can have profound importance in biology. For

* In the *trans* configuration, the two large groups of the molecule are on opposite sides of the double bond between them, and in the *cis* form they are on the same side.

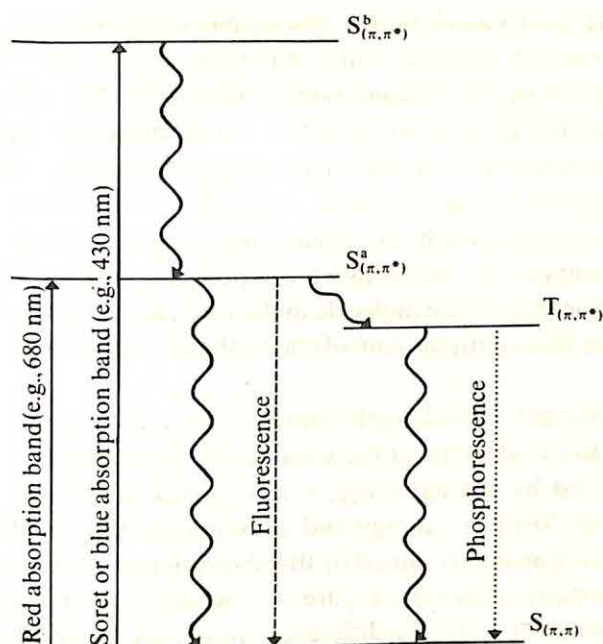


Figure 4.5

Energy level diagram indicating the principal electronic states and some of the transitions of chlorophyll. Straight vertical lines represent the absorption of light; wavy lines indicate radiationless transitions; broken lines indicate those de-excitations accompanied by radiation.

example, light can cause the photoisomerization of a *cis* form of retinal* yielding a *trans* isomer, which is the basic photochemical event triggering vision (see *Scientific American* or Wald).

Light absorption by chlorophyll. To illustrate certain of the terms introduced above to describe the absorption of light by molecules we will use chlorophyll. (Chlorophyll will be considered in more detail in Ch. 5.) The principal energy levels and electronic transitions of chlorophyll are presented in Figure 4.5. Chlorophyll is a singlet in the ground state, as are practically all pigments of importance in biology. Upon the absorption of a photon, some π electron is excited to a π^* orbital. When this excited state is a singlet,

* A carotenoid attached to the protein opsin—the complex being referred to as rhodopsin.

it is represented by $S_{(\pi, \pi^*)}$ (Figs. 4.3 and 4.5). The first symbol in the subscript is the type of electron (here a π electron) which has been excited to the antibonding orbital indicated by the second symbol (here π^*). We will represent the ground state by $S_{(\pi, \pi)}$, indicating that no electrons are in excited, or antibonding orbitals. If the orientation of the spin of the excited π electron has become reversed during excitation, it would be in the same direction as the spin of the electron which remained in the π orbital (cf. Fig. 4.3c). (Each filled orbital contains two electrons whose spins are in opposite directions.) In this case, the net spin of the molecule in the excited state would be 1, known as a triplet. The excited triplet state of chlorophyll is represented by $T_{(\pi, \pi^*)}$ in Figure 4.5.

Chlorophyll has two principal excited singlet states which differ considerably in energy (see Clayton 1965). One of these states, designated $S^a_{(\pi, \pi^*)}$ in Figure 4.5, can be excited by red light, e.g., a wavelength of 680 nm. The other state, $S^b_{(\pi, \pi^*)}$, lies higher in energy and is excited by blue light (e.g., 430 nm). The electronic transitions caused by the absorption of photons are indicated by solid vertical arrows in Figure 4.5, while the vertical distances correspond approximately to the differences in energy involved. Excitation of a singlet ground state to an excited triplet state is usually only about 10^{-5} times as probable as going to an excited singlet state, so that the transition from $S_{(\pi, \pi)}$ to $T_{(\pi, \pi^*)}$ has not been indicated for chlorophyll in Figure 4.5. In order to go from $S_{(\pi, \pi)}$ to $T_{(\pi, \pi^*)}$, the energy of the electron must be substantially increased and the orientation of the electron spin must be simultaneously reversed. Since the coincidence of these two events is rather improbable, very few chlorophyll molecules are directly excited to $T_{(\pi, \pi^*)}$ from the ground state by the absorption of light.

We will now briefly compare the two most important excitations in photobiology, viz., the transitions of n and π electrons to π^* orbitals. The n electrons have very little spatial overlap with other electrons, and they tend to be higher in energy than the π electrons, which are reduced in energy (stabilized) by being delocalized over a number of nuclei. Therefore, the excitation of an n electron to a π^* orbital generally takes less energy than the transition of a π electron to the same antibonding orbital. Hence, a $S_{(n, \pi^*)}$ state usually occurs at a lower energy—a longer wavelength is required for its excitation—than does the analogous $S_{(\pi, \pi^*)}$. Another difference is that π and π^* orbitals may overlap spatially (cf. Fig. 4.4), while n and π^* orbitals generally do not. Consequently, the n to π^* transition is not as favored or probable as the π to π^* one. Thus, transitions to $S_{(\pi, \pi^*)}$ states tend to dominate the absorption properties of a molecular species (as evidenced, for

example, by an absorption spectrum) compared with excitations yielding $S_{(n,\pi^*)}$ states.

As our final topic in this section, we will consider the effect of molecular environment on n , π , and π^* orbitals. The n electrons generally interact strongly with water, e.g., by participating in hydrogen bonding, and therefore the energy level of n electrons is considerably lower in an aqueous environment than in an organic solvent. The energy of π^* electrons is also lowered by water, but to a lesser extent than for n electrons; the π orbitals are the least affected by the solvent. Thus, a transition from an n to a π^* orbital takes more energy in water than in an organic solvent. On the other hand, the π to π^* transition takes less energy in an aqueous environment than in an organic one. As an example, let us consider the transition of chlorophyll to an excited singlet state in different solvents. The excitation to either excited singlet state takes less energy in an aqueous environment than it does in an organic solvent, i.e., when chlorophyll is in water the required photons have a longer wavelength than when the chlorophyll is dissolved in an organic solvent like acetone. This occurrence of a lower energy requirement in water is consistent with our statement that the transition for chlorophyll corresponds to the promotion of a π electron to a π^* orbital.

De-excitation

The primary processes of photochemistry involve the light absorption event, which we have already discussed, together with the subsequent de-excitation reactions. We can portray such transitions on an energy level diagram, as in Figure 4.5 for chlorophyll. In this section we will discuss the various de-excitation processes, including a consideration of their rate constants and lifetimes.

One characteristic of the various excitation and de-excitation processes is the time needed for individual transitions. For example, a useful estimate of the time it takes for the absorption of a photon is the time required for one cycle of the light wave to pass by a given electron. This time is simply the distance per cycle of the wave divided by the velocity with which light travels, or λ/ν , which equals $1/\nu$ by Equation 4.1. Hence, the time required for the absorption of a photon is approximately equal to the reciprocal of the frequency of the light, $1/\nu$, which is simply the time necessary for one complete oscillation of the electromagnetic field. During one oscillation the electric vector of light can induce an electron to move first in one direction

and then in the opposite one, thereby setting up a beating or resonating of the electron. To be specific, let us consider blue light with a wavelength of 460 nm in a vacuum. From its frequency (given in Table 4.1), we can calculate that the time for one cycle is $1/(6.52 \times 10^{14} \text{ sec}^{-1})$, or $1.5 \times 10^{-15} \text{ sec}$. Light absorption is indeed an extremely rapid event!

Times for de-excitation reactions are usually expressed in "lifetimes." A lifetime is the time required for the number of molecules in a given state to decrease to $1/e$, or 37% of the initial number. Lifetimes are extremely convenient for describing first-order processes because the initial species in such processes decay (disappear) exponentially with time (see Eq. 4.8 and also App. VI for examples of first-order processes). A "half-time," the time necessary for the number of species in a given state to decrease by 50%, is occasionally used to describe de-excitation processes—for an exponential decay, one half-time is $\ln 2$, or 0.693 times the size of a lifetime.

Fluorescence, phosphorescence, and radiationless transitions. As we would expect, the excess energy of the excited state can be dissipated by a number of competing reactions. One of the ways in which de-excitation can occur is by the emission of light known as *fluorescence* (indicated by a dashed straight line in Fig. 4.5 for the principal transitions of chlorophyll). Fluorescence describes the electromagnetic radiation emitted when a molecule goes from an excited singlet state to a singlet ground state. Fluorescent lifetimes for most organic molecules are from 10^{-9} to 10^{-6} sec (see Turro). The properties of chlorophyll fluorescence are extremely important for an understanding of the primary events of photosynthesis, and we will consider them in Chapter 5.

De-excitation of an excited state often occurs without the emission of any radiation. Such transitions are termed nonradiative, or *radiationless* (indicated by wavy lines in Fig. 4.5). For a radiationless transition to a lower energy excited state or back to the ground state, the energy of the absorbed photon is eventually converted to heat, which is passed on by collisions with the surrounding molecules. Radiationless transitions can be extremely rapid from one excited singlet state to a lower energy excited singlet state in the same molecule. For example, the radiationless transition from $S^b_{(\pi, \pi^*)}$ to $S^a_{(\pi, \pi^*)}$ indicated for chlorophyll in Figure 4.5 takes about 10^{-12} sec (see Clayton 1965). This transition is so rapid that hardly any fluorescence can be emitted from $S^b_{(\pi, \pi^*)}$, so no fluorescence emission from the upper excited singlet state of chlorophyll is indicated in Figure 4.5. The excited singlet state lying at the lower energy, $S^a_{(\pi, \pi^*)}$, can decay to the ground state by a radiationless transition. As another alternative, $S^a_{(\pi, \pi^*)}$ can go to $T_{(\pi, \pi^*)}$ also by a radiationless transition (Fig. 4.5). In fact, excited triplet states in

molecules are mainly formed by radiationless transitions from excited singlet states having higher energies. The de-excitation of $T_{(\pi, \pi^*)}$ to $S_{(\pi, \pi)}$ can be radiationless or by radiation known as *phosphorescence* (dotted straight line in Fig. 4.5), which we will consider next.

Phosphorescence is the electromagnetic radiation which can accompany the transition of a molecule from an excited triplet state to a ground state singlet. Since we go from a triplet to a singlet state, the net spin of the molecule must change during the emission of this radiation. The lifetimes for phosphorescence are usually from 10^{-3} to 10 sec (see Calvert and Pitts), which are long compared with those for fluorescence (10^{-9} to 10^{-6} sec). These relatively long times for de-excitation occur because the transition is from one electronic state to another, and the electron spin must be simultaneously reversed, the coincidence of these two events being rather improbable. In fact, the low probability mentioned above of forming $T_{(\pi, \pi^*)}$ from $S_{(\pi, \pi)}$ by light absorption has the same physical basis as the long lifetime for de-excitation of the excited triplet state back to the ground state by emitting phosphorescence. The excited triplet state of chlorophyll has not been unambiguously demonstrated *in vivo*, i.e., the expected phosphorescence is not apparent, although phosphorescence by chlorophyll *in vitro* has been shown.

Another way for an excited molecule to emit radiation is by so-called "delayed fluorescence" (see Clayton 1970). Specifically, a molecule in the relatively long-lived excited triplet state, $T_{(\pi, \pi^*)}$, can sometimes be supplied enough thermal energy by collisions to put it into the higher energy excited singlet state, $S_{(\pi, \pi^*)}$. Subsequent radiation, as the molecule goes from this excited singlet state to the ground state, $S_{(\pi, \pi)}$, has the characteristics of fluorescence. But it is considerably *delayed* after light absorption compared with normal fluorescence, since the excitation spent some time in $T_{(\pi, \pi^*)}$.

Competing pathways for de-excitation. Each excited state not only has a definite energy, it has a specific lifetime as well, the length of which depends on the particular processes competing for the de-excitation of that state. In addition to fluorescence, phosphorescence, and the radiationless transitions that we have just introduced, the excitation energy can also be transferred to another molecule, putting this second molecule in an excited state while the originally excited molecule returns to its ground state. As an example of yet another type of de-excitation process, an excited (energetic) electron may actually leave the molecule which absorbed the photon—this occurs for certain excited chlorophyll molecules which we will discuss later. The excited state which appears to be of pivotal importance in photosynthesis is the lower excited singlet state of chlorophyll, indicated

by $S_{(\pi, \pi^*)}^a$ in Figure 4.5. We will use this state to illustrate some of the possible competing reactions leading to the de-excitation of an excited singlet state, $S_{(\pi, \pi^*)}$ (see Clayton 1970; Seliger and McElroy).

The absorbed quantum can be reradiated as electromagnetic energy, $h\nu$, causing the excited molecule [$S_{(\pi, \pi^*)}$] to drop back to the ground state, $S_{(\pi, \pi)}$:



Such fluorescence exponentially decays away with time after the exciting light is removed, indicating a first-order process having a rate constant given by k_1 in Equation 4.3 (first-order rate constants have units of sec^{-1}). The fluorescent lifetime of $S_{(\pi, \pi^*)}$ is $1/k_1$, which typically is about 10^{-8} sec. (We will consider lifetimes and rate constants again below.) This fluorescent lifetime would be the actual lifetime of $S_{(\pi, \pi^*)}$, if no other competing de-excitation processes occurred. When the energy of the absorbed quantum is dissipated as fluorescence, no photochemical work can be done. Therefore, the fluorescent lifetime is essentially the upper time limit in which any biologically useful reactions can be driven by the lowest excited singlet state of a molecule.

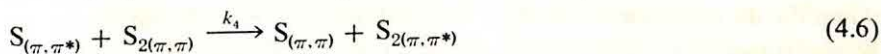
The next de-excitation processes to be considered are the radiationless transitions by which $S_{(\pi, \pi^*)}$ eventually dissipates its excess electronic energy as heat. Actually, two different states can be reached by radiationless transitions from $S_{(\pi, \pi^*)}$:



Radiationless transitions such as those indicated in Equations 4.4 and 4.5 involve de-excitations in which the excess energy is often first passed on to other parts of the same molecule. This causes excitation of certain vibrational modes for other pairs of atoms within the molecule, such as those we will shortly discuss in conjunction with the Franck-Condon principle (cf. Fig. 4.6). This energy, which has become distributed over the molecule, is subsequently frittered away by collisions with other molecules in the randomizing free-for-all interchanges that are the basis of temperature. In the case of the return of an excited molecule to its ground state by a radiationless transition (Eq. 4.4), all of the radiant energy of the absorbed light is eventually converted into the thermal energy of motion of the surrounding molecules. Some of the excess electronic energy appears in Equation 4.5 as the quantity designated "heat," and the rest represents the difference in energy between $S_{(\pi, \pi^*)}$ and $T_{(\pi, \pi^*)}$.

As in fluorescence, such radiationless transitions also obey first-order kinetics. (The rate of disappearance of the excited state by such processes, $dS_{(\pi, \pi^*)}/dt$, is linearly proportional to the amount of $S_{(\pi, \pi^*)}$ present at any time.) Although the dissipation of excitation energy as heat in a transition to the ground state (Eq. 4.4) is photochemically wasteful, in that no biological work is performed, the transition to $T_{(\pi, \pi^*)}$ indicated by Equation 4.5 can be quite useful. The lowest excited triplet state usually lasts 10^4 to 10^8 times longer than does $S_{(\pi, \pi^*)}$, which allows time for many more intermolecular collisions. Since each collision increases the opportunity for a given reaction to occur, $T_{(\pi, \pi^*)}$ can be an important excited state in photobiology.

The absorption of light can lead to a photochemical reaction initiated by a molecule other than the one which actually absorbed the photon. Such a phenomenon suggests that electronic excitation can be transferred between molecules, resulting in the excitation of one and de-excitation of the other. For instance, the excitation energy of $S_{(\pi, \pi^*)}$ might be transferred to a second molecule, represented in the ground state by $S_{2(\pi, \pi)}$:



This second molecule thereby becomes excited, indicated by $S_{2(\pi, \pi^*)}$, while the molecule which absorbed the quantum becomes de-excited and is returned to the ground state. Such transfer of electronic excitation from molecule to molecule underlies the energy migration among the pigments involved in photosynthesis (see Ch. 5). We will assume that Equation 4.6 represents a first-order reaction, as it does for the excitation exchanges between chlorophyll molecules *in vivo* (in certain cases, Eq. 4.6 can represent a second order reaction).

As another type of de-excitation process, $S_{(\pi, \pi^*)}$ can take part in a photochemical reaction. For example, the excited π^* electron can be donated to a suitable acceptor. In the following equation this ejected electron is represented by e^* :



The electron removed from $S_{(\pi, \pi^*)}$ is replaced by another donated from some other compound; $S_{(\pi)}$ in Equation 4.7 then goes back to the original ground state, $S_{(\pi, \pi)}$. (We will discuss electron energy and donation in Chs. 5 and 6.) Photochemical reactions of the form of Equation 4.7 serve as the crucial link in the conversion of radiant energy into chemical or electrical energy. Moreover, Equation 4.7 can be used to represent the photochemical reaction taking place at a special chlorophyll molecule, P_{700} , which we will discuss later.

Lifetimes and quantum yields. Equations 4.3 through 4.7 represent five competing pathways for the de-excitation of the excited singlet state, $S_{(\pi, \pi^*)}$. They must all be considered when predicting the lifetime of $S_{(\pi, \pi^*)}$. Assuming that each of these dissipative processes is first-order, we find that the disappearance of the excited singlet state satisfies the following relation:

$$-\frac{dS_{(\pi, \pi^*)}}{dt} = (k_1 + k_2 + k_3 + k_4 + k_5)S_{(\pi, \pi^*)} \quad (4.8)$$

where the various k_j s appearing in Equation 4.8 are the rate constants for the five individual decay reactions indicated (Eqs. 4.3 through 4.7). Upon integration of Equation 4.8 (see pp. 448–450, App. VI), we obtain the following expression for the time dependence of the number of molecules in the excited singlet state:

$$S_{(\pi, \pi^*)}_t = S_{(\pi, \pi^*)}_0 e^{-(k_1 + k_2 + k_3 + k_4 + k_5)t} \quad (4.9)$$

where $S_{(\pi, \pi^*)}_0$ represents the number of molecules in the excited singlet state when the illumination is ceased ($t = 0$), and $S_{(\pi, \pi^*)}_t$ is the number of excited singlet states remaining at a subsequent time t . Relations such as Equation 4.9—showing the amount of some state which remains at various times after illumination or other treatment—are extremely important for describing processes with first-order rate constants.

Since the lifetime of an excited state is the time required for the number of excited molecules to decrease to $1/e$ of the initial value, $S_{(\pi, \pi^*)}_t$ in Equation 4.9 equals $1/e S_{(\pi, \pi^*)}_0$ when t equals the lifetime τ , i.e., $S_{(\pi, \pi^*)}_\tau = e^{-1} S_{(\pi, \pi^*)}_0$, which leads to the following relationship:

$$(k_1 + k_2 + k_3 + k_4 + k_5)\tau = 1 \quad (4.10)$$

Equation 4.10 states that the greater the rate constant for *any* particular de-excitation process, the shorter will be the lifetime of the excited state.

Equation 4.10 can be generalized to include all competing reactions. This gives us the following expression for the lifetime:

$$\frac{1}{\tau} = k = \sum_j k_j = \sum_j \frac{1}{\tau_j} \quad (4.11)$$

where k_j is the first-order rate constant for the j^{th} de-excitation process and τ_j is its lifetime ($\tau_j = 1/k_j$). Also, τ is the lifetime of the excited state, while k in Equation 4.11 is the overall rate constant for its decay. Using Equation 4.11, we can re-express Equation 4.9 as follows: $S_{(\pi, \pi^*)}_t = S_{(\pi, \pi^*)}_0 e^{-kt} = S_{(\pi, \pi^*)}_0 e^{-t/\tau}$ (cf. App. VI). Equations 4.10 and 4.11 indicate that when more

than one de-excitation process is possible, τ is less than the lifetime of any individual competing reaction acting alone. In other words, the observed rate of decay of an excited state is faster than deactivation by any single competing reaction acting by itself.

If the rate constant for one particular reaction is much larger than for its competitors, the excited state will become de-excited predominantly by that process. As an example, we will consider an excited triplet state of a molecule that shows delayed fluorescence (cf. p. 187). Suppose that the lifetime for phosphorescence, τ_P , is 10^{-2} sec, in which case k_P is 100/sec. When sufficient thermal energy is supplied, $T_{(\pi, \pi^*)}$ can be raised in energy to an excited singlet state, which could then emit "delayed" fluorescence if $S_{(\pi, \pi^*)}$ decayed to the ground state by emitting electromagnetic radiation. Suppose that the rate constant for the transition from $T_{(\pi, \pi^*)}$ to $S_{(\pi, \pi^*)}$ is 20/sec. By Equation 4.1, k for these two competing pathways is $100 + 20$, or 120/sec, which corresponds to a lifetime of $1/120$, or 0.008 sec. Suppose now that another molecule, which can readily take on the excitation of $T_{(\pi, \pi^*)}$ of the original species, is introduced into the solution— k_{transfer} might be 10^4 /sec. Because of the relatively large rate constant, such a molecule "quenches" the phosphorescence and delayed fluorescence originating from $T_{(\pi, \pi^*)}$ and is generally referred to as a *quencher*. For the three pathways indicated, the overall rate constant is $100 + 20 + 10^4$, or essentially 10^4 /sec—the de-excitation here is dominated by the quencher, since $k_{\text{transfer}} \gg k_P + k_{T_{(\pi, \pi^*)} \rightarrow S_{(\pi, \pi^*)}}$.

We often use a *quantum efficiency* (quantum yield), Φ_i , to describe the de-excitation processes following absorption of light. Φ_i represents that fraction of molecules in some excited state which will decay by the i^{th} de-excitation reaction out of all possible competing pathways:

$$\begin{aligned}\Phi_i &= \frac{\text{number of molecules using } i^{\text{th}} \text{ de-excitation reaction}}{\text{number of excited molecules}} \\ &= \frac{k_i}{\sum_j k_j} = \frac{\tau}{\tau_i}\end{aligned}\quad (4.12)$$

Equation 4.12 states that the rate constant for a particular pathway determines what fraction of the molecules in a given excited state will use the i^{th} de-excitation process, hence, k_i determines the quantum yield for that de-excitation pathway. From Equation 4.11 and the definition of τ_i given above, we can also indicate such competition among pathways using lifetimes (cf. Eq. 4.12). The shorter the lifetime τ_i for a particular de-excitation pathway, the larger will be the fraction of the molecules using that pathway,

hence, the higher will be its quantum yield. Finally, by Equations 4.11 and 4.12, the sum of the quantum yields for all the competing de-excitation pathways ($\sum_i \Phi_i$) equals unity.

To illustrate the use of Equation 4.12, let us consider the quantum yield for chlorophyll fluorescence, Φ_{Fl} . The fluorescent lifetime (τ_{Fl}) of the lower excited singlet state of chlorophyll in ether is 1.5×10^{-8} sec, while the observed lifetime (τ) for de-excitation of this excited state in ether is 0.5×10^{-8} sec (see Clayton 1965). By Equation 4.12, the expected quantum yield for fluorescence would be $(0.5 \times 10^{-8} \text{ sec}) / (1.5 \times 10^{-8} \text{ sec})$, or $1/3$, which is consistent with the observed Φ_{Fl} of 0.33 for the fluorescent de-excitation of chlorophyll in ether.

Absorption spectra and action spectra

As we have discussed, the absorption of radiation causes a molecule to go from its ground state to some excited state, whereby one of the electrons enters an orbital of higher energy. We have so far considered that both the ground state and the excited states occur at *specific* energy levels, as is indicated by the horizontal lines in Figure 4.5 for the case of chlorophyll. Are only a very limited number of wavelengths absorbed? For instance, are 430 nm and 680 nm the only wavelengths absorbed by the chlorophyll molecule (Fig. 4.5)? We will find that each electronic energy level is actually divided (or split) into various sublevels which differ in energy. The largest splitting is due to *vibrational sublevels*. To help appreciate which wavelengths of light might be involved in photosynthesis or other areas of photobiology, we must know something about the vibrational sublevels which occur for a given electronic state.

Vibrational sublevels are the result of the vibration of atoms in a molecule with respect to each other. This motion determines the kinetic plus potential energy of the atoms and consequently affects the total energy of the molecule. We can describe the atomic oscillation by the accompanying change in the internuclear distance. Therefore, we will refer to an energy level diagram indicating the range of positions (trajectories) taken on by the vibrating nuclei (Fig. 4.6). The trajectories of such nuclear vibrations are quantized—i.e., only certain specific vibrations can occur, analogous to the quantization of electron spin. As a consequence of this quantization of the trajectories of the vibrating nuclei, only discrete energies are possible for the vibrational sublevels of a given electronic state. (We will consider further splitting of the vibrational sublevels later in this section.)

At temperatures normally encountered by plants, essentially all molecules are in the ground or unexcited state. Moreover, these molecules are nearly all in the lowest vibrational sublevel of the ground state—another consequence of the Boltzmann energy distribution (see p. 121). The absorption of a photon can cause a transition of the molecule from the lowest vibrational sublevel of the ground state to one of the vibrational sublevels of the excited electronic state. The actual sublevel reached depends on the energy of the absorbed quantum. For reasons that should become clear as we proceed in the discussion of vibrational sublevels, the *probability* that a photon will be absorbed also depends on its energy. Consideration of this absorption probability as a function of wavelength leads to an *absorption* spectrum for that particular molecule. The *effect*, or consequences of light absorption on some process, when presented as a function of wavelength, leads to an *action* spectrum. At the end of this section we will discuss action spectra for a phytochrome-mediated reaction.

Vibrational sublevels in an energy level diagram. Various vibrational sublevels of both ground and excited states of a molecule are schematically indicated in Figure 4.6. In principle, an energy level diagram can be prepared for any pigment, e.g., chlorophyll, carotenoid, or phytochrome. Energy level diagrams are extremely useful for predicting which electronic transitions are most likely to accompany the absorption of light. They can also be used to determine why only certain wavelengths are primarily involved.

The abscissa in Figure 4.6 is the distance between a pair of nuclei vibrating back and forth with respect to each other. The x-axis is the nuclear separation, obtained by imagining one nucleus to be situated at the origin of the coordinate system while the position of the other nucleus (in the same molecule) is plotted relative to this origin. The ordinate represents the total energy of the electrons plus that of the pair of nuclei. Figure 4.6 shows that the excited state is of course at a higher energy than the ground state and that the two electronic states are split into many vibrational sublevels differing in energy (see Seliger and McElroy, or Turro).

There are restrictions on the range of vibration of nuclei. Internuclear and interelectronic repulsive forces act against the electrostatic attraction between nuclei and shared electrons, so that only a certain range of internuclear separation can occur for a particular bond in a molecule. As internuclear separation decreases, the nuclei repel each other more and more, and the energy of the molecule increases. Moreover, clouds of negative charge representing electrons localized on each nucleus have a greater overlap as internuclear separation decreases, resulting in interelectronic repulsion and therefore an increase in molecular energy. These effects account for the steep rise of the energy curves in Figure 4.6 as nuclear

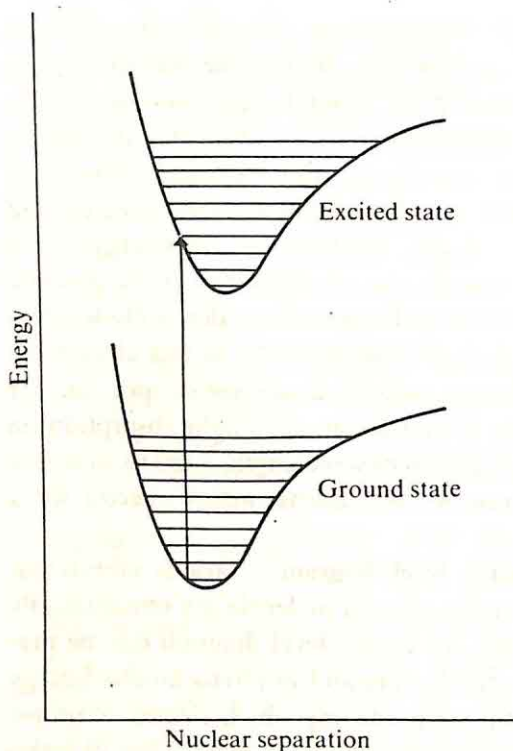


Figure 4.6

Energy curves for the ground state and an excited state showing the various vibrational sublevels. The vertical arrow represents a transition which is caused by the absorption of a photon and which is consistent with the Franck-Condon principle.

separation becomes less (left side of the figure). On the other hand, the delocalized (bonding) electrons shared by the two nuclei resist an unlimited increase in nuclear separation, which would diminish the attractive electrostatic interaction between nuclei and electrons. (Such an increase in internuclear distance corresponds to a stretching of the chemical bond.) Thus, the energy curve in Figure 4.6 also rises as the internuclear distance becomes greater (right side of the figure). Because of these two opposing tendencies, the range of possible nuclear separations is confined to an *energy trough*. It is within these potential energy curves—one for the ground state and another for the excited state—that the trajectories of the nuclear vibrations occur.

A horizontal line in Figure 4.6 represents the actual range of nuclear separations corresponding to a given vibrational sublevel, i.e., nuclei vibrate back and forth along the distance indicated by a horizontal line. As is evident in the figure, both ground and excited states have many vibrational sublevels which differ in energy. For the upper vibrational sublevels of a given electronic state, the nuclei vibrate over longer distances (i.e., more extensive range of nuclear separations in Fig. 4.6), which also corresponds to higher vibrational energies. Since the excited state has an electron in an antibonding orbital, it usually has a greater mean internuclear separation than does the ground state. This increase in bond length is shown in Figure 4.6 by a slight displacement to greater nuclear separations, i.e., to the right, for the upper curve.

Because the direction of nuclear motion is reversed at the extremities of the vibrational pathways (horizontal lines in Fig. 4.6), the velocity of the nuclei must be zero at these turning points. As the turning point at either end of the oscillation range is approached, the nuclei begin to slow down and eventually stop before reversing their direction of motion. Consequently, the nuclei spend most of their time at or near the extreme ends of their trajectory. A photon is most likely to arrive at the molecule when the nuclei are at or near the extremes of their vibrational range.* Because of our probability consideration, the electronic transition resulting from the absorption of a photon (the vertical arrow in Fig. 4.6) has been initiated from one of the ends of the nuclear oscillation range for the lowest vibrational sublevel of the ground state. The arrow in Figure 4.6 begins from the lowest sublevel because nearly all ground state molecules are in the lowest vibrational sublevel at the temperatures encountered in plants, reflecting the Boltzmann energy distribution.

During the light absorption event, the energy of the photon is transferred to some electron in the molecule. Since the molecular orbital describing the trajectory of an electron in the excited state has a small, but finite probability of spatially overlapping with the nuclei, an interaction is possible between the excited electron and the nuclei over which it is delocalized. Such interactions do occur and generally take place over a rather short time period (less than 10^{-13} sec). An interaction between an energetic electron and the nuclei can cause the excitation of nuclei to higher energy vibrational states. In fact, the transition represented by the arrow in Figure 4.6 corresponds

* Actual quantum-mechanical calculations beyond the scope of this text lead to somewhat different conclusions, especially for the lowest vibrational sublevel. See Calvert and Pitts, or Turro.

to both the excitation of an electron leading to an excited state of the molecule and the subsequent excitation of the nuclei to some excited vibrational sublevel. Thus, part of the energy of the light quantum is rapidly passed on to nuclear vibrations. The length of the arrow in Figure 4.6 is proportional to the light energy (or quantum) actually added to the molecule, and therefore represents the energy distributed to the nuclei plus that remaining with the excited electron. Next, we will demonstrate which vibrational sublevel of the excited state has the highest probability of being reached by the excitation process.

The Franck-Condon principle. Franck and Condon in 1926 enunciated a principle, based mainly on classical mechanics, to help rationalize the various bands in absorption spectra and fluorescence emission spectra. Although it is the electrons which take part in the absorption of a photon, we will direct our attention to the nuclei in order to discuss the effect of the quantized modes of nuclear vibration, and will therefore use the energy level diagram presented in Figure 4.6. The Franck-Condon principle states that the nuclei change neither their *separation* nor their *velocity* during those transitions for which the absorption of a photon is most probable (see Seliger and McElroy, or Turro). We can use this principle in order to predict which vibrational sublevels of the excited state are most likely to be involved in the electronic transitions accompanying light absorption.

First, we will examine that part of the Franck-Condon principle which states that nuclei do not change their position for the most probable electronic transitions caused by light absorption. Since a vertical line in Figure 4.6 corresponds to no change in nuclear separation, the absorption of a photon has been indicated by a vertical arrow. This condition of constant internuclear distance during absorption can be satisfied more often when the nuclei are moving slowly or actually stopped at the extremes of their oscillation range. Thus, the origin of the arrow indicating an electronic transition in Figure 4.6 is at one of the turning points of the lowest vibrational sublevel of the ground state, and the tip is drawn to an extremity of one of the vibrational sublevels in the excited state (the fourth vibrational sublevel for the particular case illustrated).

The other condition embodied in the Franck-Condon principle is that a quantum has the greatest chance of being absorbed when the velocity (a vector) of the vibrating nuclei does not change. In other words, absorption would be maximal when the nuclei are moving in the same direction and at the same speed in both ground and excited states. Again, this condition has the greatest statistical probability of being met when the nuclei are moving slowly or not at all, as occurs at the turning points for nuclear

oscillation. Therefore, the most probable electronic transition represented in a diagram like Figure 4.6 is a vertical line, which originates from one of the ends of the horizontal line representing the range of nuclear separations for the lowest vibrational sublevel of the ground state and goes to the end of the vibrational trajectory for some sublevel of the excited state.

Another way to view the Franck-Condon principle is to consider that the light absorption event is so rapid that the nuclei do not have a chance to move during it. The absorption of a photon requires about 10^{-15} sec. By contrast, the period for one nuclear vibration back and forth along an oscillation range (like those represented by horizontal lines in Fig. 4.6) is generally somewhat more than 10^{-13} sec. Thus, the nuclei cannot move any appreciable distance during the time necessary for the absorption of a photon, especially when the nuclei are moving relatively slowly near the ends of their vibrational trajectory. Moreover, nuclear velocity would not change appreciably in 10^{-15} sec (see Turro).

Since we have discussed the frequency of nuclear oscillations, we should note another aspect with far-reaching consequences. As the nuclei oscillate back and forth along their trajectories, they can have collisions with other nuclei. These encounters make possible the transfer of energy from one nucleus to another (within the same molecule or to adjacent molecules). Thus, the time for one cycle of a nuclear vibration, approximately 10^{-13} sec, is an estimate of the time in which excess vibrational energy can be dissipated as heat by interactions with other nuclei. As excess energy is exchanged by such processes, the part of the molecule indicated in Figure 4.6 soon reaches the lowest vibrational sublevel of the excited state; these transitions within the same electronic state, e.g., $S(\pi, \pi^*)$, are usually complete in about 10^{-12} sec. Fluorescence lifetimes generally are on the order of 10^{-8} sec, so that an excited singlet state gets to its lowest vibrational sublevel before appreciable de-excitation can occur by fluorescence. The rapid dissipation of excess vibrational energy causes some of the energy of the absorbed photon to be released as heat. Therefore, fluorescence is generally of lower energy (longer wavelength) than the absorbed light, as we will show for chlorophyll in the next chapter.

The Franck-Condon principle predicts the most likely electronic transition caused by the absorption of light. However, others do occur. These other transitions become statistically less probable, the further the conditions are from no change in nuclear position or velocity during the absorption of the photon. Since electronic transitions to both higher and lower vibrational sublevels in the excited state occur with a lower probability than transitions to the optimal sublevel, the absorption of light is not as great at the wave-

lengths which excite the molecule to such vibrational sublevels. Moreover, some transitions begin from an excited vibrational sublevel of the ground state. We can calculate the fraction of the ground state molecules in the various vibrational sublevels from the Boltzmann energy distribution, $n(E) = n_{\text{total}} e^{-E/kT}$ (Eq. 3.21). For example, $e^{-E/kT}$ is 0.022 for an E of 0.1 ev at 25°, as we calculated on p. 169.

What is the energy spacing between vibrational sublevels? For many molecules the vibrational sublevels of both the ground and the excited states are approximately 0.1 ev apart. Consequently, as the wavelength of incident light is increased or decreased from that for the most intense absorption, transitions involving other vibrational sublevels become important, e.g., at approximately 0.1 ev intervals.* This can be seen by the various peaks near the major absorption bands of chlorophyll (Fig. 5.2) or the three peaks in the absorption spectra of typical carotenoids (Fig. 5.4). In summary, the amount of light absorbed will be maximal at a certain wavelength corresponding to the most probable transition predicted by the Franck-Condon principle. Transitions from other vibrational sublevels of the ground state and to other sublevels of the excited state occur less frequently and help create an absorption spectrum that is characteristic of a particular molecule.

Absorption bands and absorption coefficients. Our discussion of light absorption has so far been primarily concerned with transitions from the ground state energy level to those of excited states, which we just expanded to include the occurrence of vibrational sublevels of the electronic states. In addition, vibrational sublevels are subdivided into *rotational* states. In particular, the motion of the atomic nuclei within a molecule can be described by quantized rotational states of specific energy, leading to the subdivision of a given vibrational sublevel of an electronic state into a number of rotational subsublevels. The energy increments between rotational states are generally about 0.01 ev (approximately 3 nm in wavelength in the visible region). The further broadening of absorption lines because of a continuum of *translational* energies of the whole molecule is generally much less, often about 0.001 ev. Moreover, interactions with the solvent or other neighboring molecules can affect the distribution of electrons in a particular molecule and consequently can shift the position of the various energy levels. The magnitude of the shifts caused by intermolecular inter-

* An energy difference of 0.1 ev between two photons corresponds to a difference in wavelength of about 25 nm near the middle of the visible region of the spectrum (green or yellow, Table 4.1).

actions vary considerably and can be 0.05 eV or more (see Depuy and Chapman; Jaffé and Orchin; Moore; Seliger and McElroy).

The quanta absorbed in a specific electronic transition—including the range of energies due to the various rotational subsublevels and other shifts—give rise to an absorption *band*. This is a range of wavelengths that can be absorbed for the transition of an electron from a vibrational sublevel of the ground state to some vibrational sublevel of an excited state. A plot of the relative efficiency for light absorption as a function of wavelength is an absorption *spectrum*, which may include more than one absorption band. Such bands can represent transitions to or from different vibrational sublevels and to different excited states. The smoothness of the absorption bands of most pigment molecules indicates that a great number of different photon energies can correspond to the transition of an electron from the ground state to some excited state (cf. Figs. 4.9, 5.2, 5.4, and 5.6).^{*} Because of the rather large effects intermolecular interactions can have on electronic energy levels, we should always specify the solvent when presenting an absorption band or spectrum.

Whenever we deal with absorption bands or spectra, we are concerned with how the light absorption varies with wavelength. The absorption at a particular wavelength by a certain species is quantitatively described using an *absorption coefficient*, ϵ_λ . (ϵ_λ is also referred to as an *extinction coefficient*.) Because of its descriptive usefulness in plant and cell physiology, we will derive an expression incorporating ϵ_λ .

Let us consider a monochromatic beam of parallel light of intensity I_0 . Since monochromatic means “of a single wavelength,” the “intensities” referred to here can be expressed as either photon or energy fluxes. While passing through a solution some of the light may be absorbed, and so the emerging beam will generally have a lower intensity, I_t —we will assume that scattering and reflection are negligible. In a small path length dx along the direction of the beam, the light intensity I decreases by an amount dI due to absorption by a species having a concentration c . Lambert is often credited with recognizing in 1768 that $-dI/I$ was proportional to dx —ac-

^{*} To “sharpen” an absorption spectrum, it is sometimes experimentally advantageous to reduce the translational broadening of absorption bands by substantially decreasing the temperature, e.g., by using liquid nitrogen (boiling point = -196°) to cool the sample. Also, the reduction in temperature decreases the number of the absorbing molecules in excited vibrational sublevels and higher energy rotational subsublevels—we can predict the relative populations of these states from the Boltzmann factor, $e^{-E/kT}$, Equation 3.21.

tually, Bouguer had expressed this fact in 1729—and in 1852 Beer noted that $-dI/I$ was proportional to c . Upon putting these two observations together, we obtain the following expression:

$$-\frac{dI}{I} = k_{\lambda}cdx \quad (4.13)$$

where k_{λ} is a proportionality coefficient referring to a particular wavelength, and the minus sign occurs because the light intensity is of course decreased by absorption. After dividing both sides by I , we can integrate Equation 4.13 across a solution of a given concentration, which leads to

$$\int_{I_o}^{I_f} -dI/I = -\ln \frac{I_f}{I_o} = \int_0^b k_{\lambda}cdx = k_{\lambda}cb \quad (4.14)$$

where I_o is the intensity of the incident beam, and I_f is its intensity after traversing a distance b through the solution.

The usual practice is to recast Equation 4.14 into a slightly more convenient form. We can replace the natural logarithm by the common logarithm ($\ln = 2.303 \log$, App. III), and $k_{\lambda}/2.303$ by the absorption coefficient at a specific wavelength, ϵ_{λ} . Equation 4.14 then becomes

$$A_{\lambda} = \log \frac{I_o}{I_f} = \epsilon_{\lambda}cb \quad (4.15)$$

where A_{λ} is the absorbance (colloquially, the “optical density”) of the solution at the particular wavelength being considered. Equation 4.15 is generally referred to as Beer’s law, although it is also called the Beer-Lambert law, the Lambert-Beer law, and even the Bouguer-Lambert-Beer law. When more than one absorbing species is present in a solution, we can generalize Equation 4.15 to give

$$A_{\lambda} = \log \frac{I_o}{I_f} = \sum_j \epsilon_{\lambda_j}c_jb \quad (4.16)$$

where c_j is the concentration of species j , and ϵ_{λ_j} is its absorption coefficient at wavelength λ (see Calvert and Pitts; Clayton 1970; Dyer; Jaffé and Orchin; and van Holde for additional information).

According to Beer’s law, the absorbance at some particular wavelength is proportional to the concentration of the absorbing species and to its absorption coefficient at that wavelength. In many absorption studies performed in the laboratory using solutions of biological pigments, the optical

path length b is 1 cm, and the absorbing species is dissolved in a solvent that does not absorb light at the wavelengths under consideration. The concentration of the absorbing species (c) is usually expressed in moles/cm³ or moles/liter, hence the absorption coefficient ϵ_λ is in cm²/mole or liters/mole-cm, respectively. When expressed in liters/mole-cm, ϵ_λ is called the molar absorption coefficient (also referred to as the molar extinction coefficient). Values of ϵ_λ for organic compounds range from less than 1 to over 10⁵ liters/mole-cm in the visible region. If ϵ_λ at some wavelength is known for a particular species, we can determine its concentration from the measured absorbance at that wavelength by using Beer's law (Eq. 4.15).

As an example of the application of Beer's law, we will estimate the average chlorophyll concentration in leaf cells. The palisade and spongy mesophyll cells in the leaf section portrayed in Figure 1.2 might correspond to an average thickness of chlorophyll-containing cells of about 200 μ m (0.02 cm). The maximum molar absorption coefficient (ϵ_λ) in the red or the blue bands of chlorophyll (cf. Figs. 4.5 and 5.2) is close to 10⁵ liters/mole-cm. At the peaks of the absorption bands about 99% of the incident red or blue light can be absorbed by chlorophyll in a leaf section like the one illustrated in Figure 1.2. This corresponds to having an emergent intensity (I_t) equal to 1% of the incident intensity (I_o), so that the absorbance (A_λ) in Equation 4.15 equals $\log(100/1)$ or 2—for simplicity, we are ignoring absorption by pigments other than chlorophyll. Using Beer's law, we find that the average chlorophyll concentration (c) is $(2)/[10^5 \text{ liters/mole-cm}(0.02 \text{ cm})]$, or 1×10^{-3} mole/liter, a value characteristic of the average chlorophyll concentration in the photosynthesizing cells in many leaves.

Chlorophyll is located only in the chloroplasts, which in turn occupy about 4%, or $1/25$, of the volume of a mesophyll cell in the leaf of a higher plant. The average concentration of chlorophyll in chloroplasts is thus about twenty-five times higher than the above estimate of chlorophyll in a leaf, or approximately 25 mM. A typical light path parallel to the minor axis or thickness of a chloroplast (cf. Fig. 1.9) is about 2 μ m (2×10^{-4} cm). Again using Beer's law ($A_\lambda = \epsilon_\lambda cb$), we find that the absorbance of a single chloroplast in the red or blue bands is about $(10^5 \text{ liters/mole-cm})(25 \times 10^{-3} \text{ mole/liter})(2 \times 10^{-4} \text{ cm})$, or 0.50. This means that I_o/I_t equals 3.16— $\log(3.16) = 0.500$, using the log table in Appendix V—so I_t is only about 32% of I_o . Therefore, approximately 68% of the incident red or blue light at the peak of absorption bands is absorbed by a single chloroplast, which helps explain why individual chloroplasts appear rather green under a light microscope.

Conjugation. Light absorption by molecules generally involves transi-

tions of π electrons to excited states where the electrons are in π^* orbitals. These π electrons occur in double bonds; the more double bonds in some molecule, the greater is the probability for light absorption by that species. Both the effectiveness in absorbing electromagnetic radiation and the wavelengths involved are determined by the number of double bonds in *conjugation*, where conjugation refers to the alternation of single and double bonds (e.g., $\text{C}=\text{C}-\text{C}=\text{C}-\text{C}=\text{C}-\text{C}$) along some part of the molecule. As the number of double bonds in the conjugated system increases, the absorption bands shift to longer wavelengths and they become more intense. It should be no surprise that the height of a band increases with the number of double bonds in conjugation. The absorption coefficient (ϵ_λ) increases with the number of double bonds in the molecule simply because there are then more delocalized (shared) π electrons capable of interacting with light.

To help understand why the number of double bonds in conjugation affects the wavelength position for an absorption band, let us consider the shifts in energy for the various orbitals as the number of π electrons in the conjugated system changes. The various π orbitals in a conjugated system

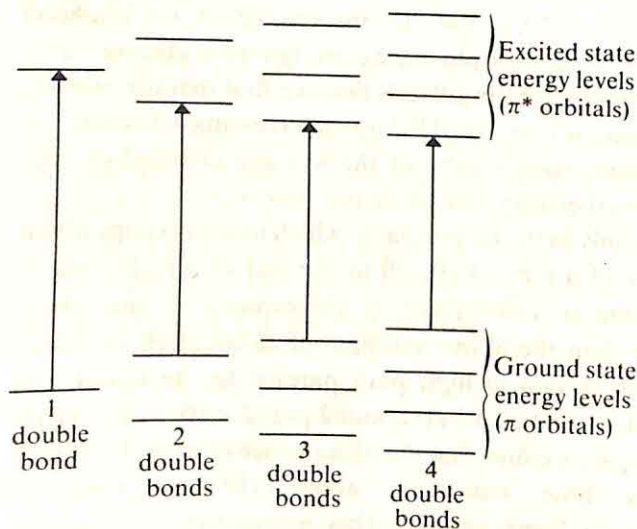


Figure 4.7

Effect on the energy levels of π and π^* orbitals as the number of double bonds in conjugation increases. The vertical arrows represent transitions caused by the absorption of photons—the required energy becoming less and less as the extent of conjugation increases.

occur at different energy levels, the average energy remaining about the same as for the π orbital in an isolated double bond not part of a conjugated system (Fig. 4.7). The more double bonds in the conjugated system, the more π orbitals there are in the conjugated system, and the greater is the energy range from the lowest to the highest energy π orbital (see Fig. 4.7). Since the *average* energy of the π orbitals in a conjugated system does not markedly depend on the number of double bonds, the energy of the highest energy π orbital increases as the number of double bonds in conjugation increases. The π^* orbitals are similarly split into various energy levels (diagrammed in Fig. 4.7). Again, the range of energy levels about the mean for these π^* orbitals increases as the number of double bonds in the conjugated system increases. Consequently, the more π^* orbitals that are available in the conjugated system, the lower in energy will be the lowest of these (see Calvert and Pitts, Clayton 1970, and Dyer).

In Figure 4.7 we present transitions from the highest energy π orbital to the lowest energy π^* orbital for a series of molecules differing in the number of double bonds in conjugation. We immediately notice that the more delocalized π electrons there are in the conjugated system, the less energy is required for a transition. This is illustrated in Figure 4.7 by a decrease in the length of the vertical arrow (which represents an electronic transition) as the number of double bonds in conjugation increases. Moreover, the most likely or probable electronic transition in this case is the one involving the least amount of energy, i.e., the excitation from the highest energy π orbital to the lowest π^* orbital is actually the predominant one.*

The decrease in energy separation between the π orbitals and the π^* orbitals as the number of double bonds in conjugation increases (Fig. 4.7) accounts for the accompanying shift of the peaks of the absorption bands toward longer wavelengths. For example, an isolated double bond ($\text{C}=\text{C}$) generally absorbs maximally near 185 nm in the ultraviolet and has a maximum absorption coefficient of nearly 10^4 liters/mole-cm. For two double bonds in conjugation ($\text{C}=\text{C}-\text{C}=\text{C}$), the absorption coefficient doubles and the wavelength position for maximum absorption shifts to about 225 nm, i.e., toward the visible region and longer wavelengths. As the number of double bonds in conjugation in straight-chain hydrocarbons increases from 3 to 5 to 7 to 9, the center of the absorption band

* The transition probability depends on the spatial overlap between the quantum-mechanical wave functions describing the trajectories of the electrons in the two states, and such overlap is relatively large between the highest energy π orbital and the lowest energy π^* one. See Orchin and Jaffé or Sandorfy.

for these hydrocarbons when they are dissolved in hexane is shifted from approximately 265 to 325 to 375 to 415 nm, respectively (see Calvert and Pitts as well as Levine). The absorption coefficient is approximately proportional to the number of double bonds in the conjugated system, hence it increases to almost 10^5 liters/mole-cm for the hydrocarbon containing 9 double bonds in conjugation. For molecules to absorb strongly in the visible region, a fairly extensive conjugated system of double bonds is generally necessary, as is indeed the case for pigments such as the chlorophylls and the carotenoids discussed in the next chapter.

Action spectra. The relative *effectiveness* of various wavelengths in producing a specified response is of basic importance in photobiology and leads to an *action spectrum*. An action spectrum is complementary to an absorption spectrum, the latter being the relative probability for the absorption of different wavelengths, e.g., ϵ_λ versus λ . When many different types of pigments are present, the action spectrum for a particular response can differ greatly from the absorption spectrum of the entire system. However, the Grotthus-Draper law cited on p. 175—only absorbed light leads to a photochemical reaction—implies that an action spectrum should resemble the absorption spectrum of the particular substance which absorbs the light responsible for the specific effect or action being considered.

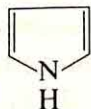
In order to obtain an action spectrum for some particular response, we could expose the system to the same number of photons per unit area per unit time at each of a series of wavelength intervals and measure the resulting effect or action. The action could be the amount of oxygen evolved, fraction of seeds germinating, or some other measured change. We could then plot the responses obtained as a function of their respective wavelength intervals in order to see which wavelengths are most effective in leading to that "action" (cf. Fig. 5.8). Another way to obtain an action spectrum is to plot the reciprocal of the number of photons required in the various wavelength intervals to give a particular response. If twice as many photons are needed at one particular wavelength compared with a second, the action spectrum would have half the height at the first wavelength—thus, the effectiveness of various wavelengths can easily be presented (cf. Fig. 4.10). Using the latter approach, the photon flux is varied until the response is the same at each wavelength interval. This is an important point; if it is to be a true action spectrum, the action or effect measured must be linear with photon flux at each of the wavelength intervals used, i.e., we must not be approaching light saturation. When we approach light saturation at certain wavelengths, the measured action per photon is less than it should be, compared with the values at other wavelengths. Consequently, an action

spectrum, such as Figure 5.8, is flattened for those wavelengths where we are approaching light saturation. In the extreme case of light saturation at *all* wavelengths, the action spectrum plotted in Figure 5.8 is perfectly flat, since the response is then the same at each wavelength (see Clayton 1970, or Seliger and McElroy).

We can compare the action spectrum of some response with the absorption spectra of the various pigments suspected of being involved in that process in order to see whether one type of pigment is responsible. If the measured action spectrum closely resembles the known absorption spectrum of some molecule, light absorbed by that molecule may be leading to the particular action we are considering. Examples where the use of action spectra have been important in understanding the photochemical aspects of plant physiology include the study of photosynthesis (Ch. 5) and investigations of the responses mediated by the pigment phytochrome, which we now turn to.

Absorption and action spectra of phytochrome. The important plant pigment phytochrome is responsible for the regulation of many aspects of plant growth and development, such as seed germination, formation of certain pigments, stem elongation, leaf expansion, and photoperiodism (see Mohr). We will direct our attention first to the proposed structures of two forms of phytochrome and then to their absorption spectra. The absorption spectra will subsequently be compared with the action spectra obtained for the promotion as well as for the inhibition of seed germination.

The tentative structures for two interconvertible forms of phytochrome are given in Figure 4.8.* Phytochrome is a tetrapyrrole, as are the chlorophylls and the phycobilins which we will discuss in the next chapter. *Pyrrole* refers to a five-membered ring having 4 carbons, 1 nitrogen, and 2 double bonds,



The forms of phytochrome designated P_{660} and P_{730} are both highly conjugated, as the structures in Figure 4.8 indicate (P stands for pigment). The P_{660} form has 7 double bonds in conjugation. Not all the double bonds in P_{660} are in the conjugated system, since only those alternating with single bonds along the molecule are part of the main conjugation. For instance, for P_{660} in Figure 4.8, the double bond at the top of the pyrrole ring second

*Strictly speaking, the term *phytochrome* applies to the phytochrome pigment, or chromophore (Fig. 4.8), plus the protein to which it is attached. However, the chromophore, or light-absorbing part, alone is loosely referred to as phytochrome.

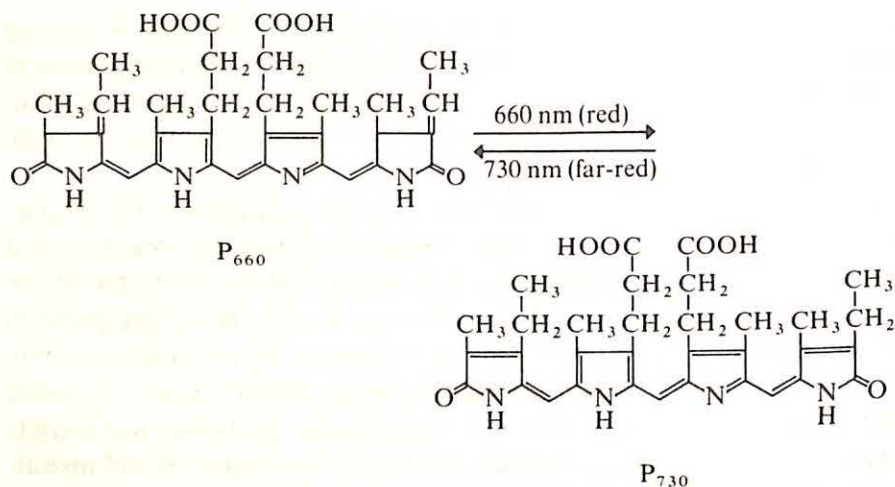


Figure 4.8

Tentative structures of interconvertible forms of phytochrome which absorb strongly in the red region (P_{660}) and in the far-red (P_{730}). (These structures are taken from H. W. Siegelman, D. J. Chapman, and W. J. Cole, in *Porphyrins and Related Compounds*, Biochemical Society Symposium No. 28, T. W. Goodwin, ed., Academic Press, London, 1968, pp. 107-120. Used by permission.)

from the right is a branch, or cross-conjugation to the main conjugation and only slightly affects the wavelength position for maximum absorption. On the other hand, the bonds in the pyrrole on the far right on P_{660} in Figure 4.8 have partial double bond character when all the possible resonating structures are considered, so they tend to increase somewhat the extent of conjugation.* The P_{730} form given in Figure 4.8 has a total of 10 double bonds in the conjugated system, including the 2 double bonds in the carbonyl groups on either side of the chromophore. On the basis of the extensive conjugation, we would expect phytochrome to absorb in the visible region, as in fact it does.

The absorption spectra of two forms of phytochrome (structures in Fig. 4.8) are presented in Figure 4.9. The structure having the greater extent of conjugation, P_{730} , has absorption bands at longer wavelengths than is the case for the other form, P_{660} . We could have predicted this from the effect of the number of conjugated double bonds on the wavelength position

* There are semiempirical rules for predicting the wavelength position of maximum absorption based on all double bonds which occur, including those in branches to the main conjugated system. See Calvert and Pitts; Dyer; or Jaffé and Orchin.

for maximum absorption discussed in the preceding section. The subscript in P_{660} indicates that this form of phytochrome has a peak absorption in the red near 660 to 665 nm (Fig. 4.9). However, the absorption band is rather broad, and many different wavelengths of light can be absorbed by P_{660} . The other form of phytochrome we are considering has a broad band extending into the near infrared (also Fig. 4.9). Absorption is maximal near 725 to 730 nm, and the structure is therefore designated P_{730} . As is indicated in Figure 4.9, the maximum molar absorption coefficients are about 10^5 liters/mole-cm for both P_{660} and P_{730} . The plant pigments we will consider in the next chapter have similar high values of ϵ_λ . Finally, the absorption spectra for P_{660} and P_{730} overlap considerably, e.g., 660 nm light can be readily absorbed by either pigment.

Next, we will examine the action spectra for seed germination responses (Fig. 4.10) with regard to the known absorption properties of phytochrome. In green tissues chlorophyll absorption in the red region markedly affects the amount of light actually incident on phytochrome, which makes the evaluation of action spectra more difficult. Consequently, we have chosen germination responses of seeds relatively free of chlorophyll to illustrate a possible phytochrome-mediated reaction. Figure 4.10 indicates that a pig-

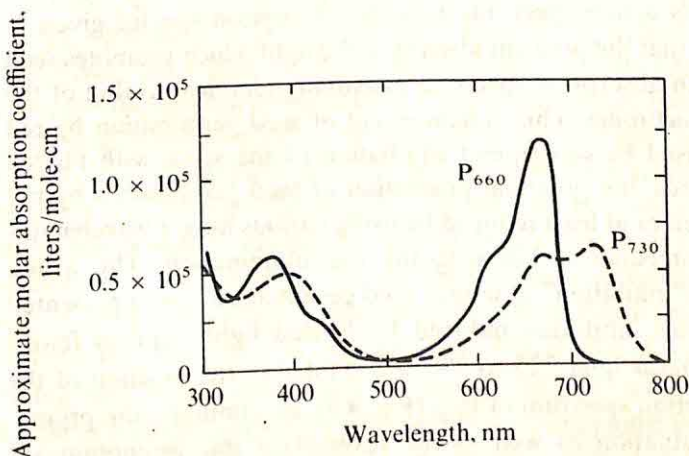


Figure 4.9

Absorption spectra for the red (P_{660}) and the far-red (P_{730}) absorbing forms of phytochrome. (Data are replotted from W. L. Butler, S. B. Hendricks, and H. W. Siegelman, in *Chemistry and Biochemistry of Plant Pigments*, T. W. Goodwin, ed., Academic Press, London, 1965, pp. 197-210. Used by permission.)

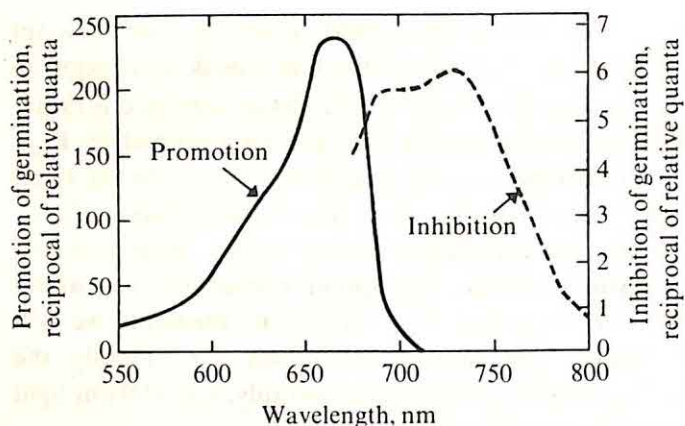


Figure 4.10

Action spectra for the promotion of lettuce seed germination and its reversal, or "inhibition." (Data are replotted from S. B. Hendricks and H. A. Borthwick, in *Chemistry and Biochemistry of Plant Pigments*, T. W. Goodwin, ed., Academic Press, London, 1965, pp. 405-436. Used by permission.)

ment absorbing in the red region promotes the germination of lettuce seeds. If we compare this action spectrum with the absorption spectra given in Figure 4.9, we see that the pigment absorbing the light which promotes seed germination has an absorption spectrum essentially identical to that of the P_{660} form of phytochrome. This enhancement of seed germination by red light can be reversed by subsequent irradiation of the seeds with far-red light. In other words, the potential promotion of seed germination by red light can be stopped or at least reduced by using various longer wavelengths for irradiation immediately following the red illumination. The action spectrum for such "inhibition" of lettuce seed germination is also presented in Figure 4.10. This inhibition induced by far-red light requires fewest quanta at wavelengths near 725 to 730 nm, similar to the position of the peak in the absorption spectrum of P_{730} (Fig. 4.9). In summary, the promotion of seed germination as well as the reversal of this promotion are apparently controlled by two forms of phytochrome, P_{660} and P_{730} , which can be reversibly interconverted by light.

The physiological significance of phytochrome interconversions lies in the fact that P_{730} is the active form. Light absorbed by P_{660} leads to its conversion to P_{730} , and this latter form promotes the germination of the lettuce seeds. If far-red light is incident on the tissue, its absorption by P_{730} tends to convert

phytochrome to the inactive P_{660} form, and the germination is not promoted. Let us consider a seed present on the surface of the soil under a dense canopy of leaves. Because of their chlorophyll, leaves absorb red light preferentially to far-red, so that very little red light compared with far-red would reach the seed. Consequently, the phytochrome in the seed would occur predominantly in the P_{660} form. However, if this seed were moved into a patch of sunlight, a larger fraction of the P_{660} would be in the active form, P_{730} , and seed germination could proceed under illumination conditions favorable for photosynthesis and thus for growth.

Action spectra studies have indicated that on a quantum basis the maximum sensitivity is usually quite different for the opposing responses attributable to the two forms of phytochrome. (Since the expression of phytochrome action involves a multistep process including biochemical reactions, there is no reason why the two responses should have the same sensitivity.) For the example of lettuce seed germination, about 30 times more far-red quanta (e.g., at 730 nm) are required to cause a 50% inhibition compared with the number of red quanta (e.g., at 660 nm) needed to promote seed germination by 50%—compare the different scales used for the ordinates in Figure 4.10. Thus, ordinary sunlight is physiologically equivalent to red light, as much of the phytochrome is converted to the active form, P_{730} .

Another important aspect of the role of phytochrome in plants is that the P_{730} form reverts spontaneously to P_{660} over a period of hours in the dark. Thus, the length of the night becomes an important variable for photoperiodic responses affected by P_{730} . In particular, phytochrome interconversions provide a plant with a timing mechanism for distinguishing between long nights and short nights, consequently, certain physiological responses regulated by the P_{660} - P_{730} system can be initiated at the appropriate time or season during the year. The interconversions of phytochrome indicated in a simplified fashion in Figure 4.8 are actually multistep processes involving a number of intermediates whose structures and absorption characteristics are under investigation. Moreover, the electronic transitions and the biochemical mechanisms of action of phytochrome are not yet fully understood. Finally, as we might expect, individual plants vary considerably in how they amplify and use the information provided by the P_{660} - P_{730} system. (See Briggs and Rice; Mitrakos and Shropshire, or Salisbury and Ross for further information on phytochrome.)

Our discussion of phytochrome leads us to the concept of a *photostationary state*. A photostationary state refers to the relative amounts of interconvertible forms of some pigment which occur in response to a particular steady-illumination condition—such a state might have been called, more

appropriately, a "photosteady" state. When illumination is constant, the conversion of P_{660} to P_{730} will eventually achieve the same rate as the reverse reaction (cf. Fig. 4.8). The ratio of P_{660} to P_{730} for this photostationary state depends on the absorption properties of each form of the pigment for the incident wavelengths (note that the absorption spectra overlap, Fig. 4.9), the number of photons in each wavelength interval, and the kinetics of the competing de-excitation reactions. If the light quality and/or quantity were to change to another constant condition, we would shift to a new photostationary state where P_{660}/P_{730} would in general be different. Since the ratio of P_{660} to P_{730} determines the overall effect of the phytochrome system, a description in terms of photostationary states is useful for discussing the influence of this pigment.

Problems

- 4.1** Consider electromagnetic radiation with the indicated wavelengths in a vacuum. (a) If λ is 4000 Å, how much energy in eV is carried by 10^5 photons? (b) If an Einstein of 1800 nm photons is absorbed by a liter of water at 0°, what would be the final temperature? (Assume that there are no other heat exchanges with the external environment.) (c) A certain filter, which passes all wavelengths below 600 nm and absorbs all those above 600 nm, is placed over a radiometric device. If the meter indicates 100 ergs/cm²-sec, what is the maximum flux in photons/cm²-sec? (d) What is the illumination in (c) expressed in lux (lumens/m²)?
- 4.2** Consider electromagnetic radiation having a frequency of 0.9×10^{15} cycles/sec. (a) The velocity of the radiation is 2.0×10^8 m/sec in dense flint glass. What are the wavelengths in a vacuum, in air, and in such glass? (b) Can such radiation cause an $S_{(\pi, \pi)}$ ground state to go directly to $T_{(\pi, \pi^*)}$? (c) Can such radiation cause the transition of a π electron to a π^* orbital in a molecule having six double bonds in conjugation? (d) Electromagnetic radiation is often expressed in "wave numbers," which is the frequency divided by the velocity of light in a vacuum, i.e., ν/c , which is $1/\lambda_{\text{vacuum}}$. What is the wave number in cm⁻¹ in the present case?
- 4.3** Suppose that the quantum yield for ATP formation—molecules of ATP formed/number of excited chlorophyll molecules—is 0.40 at 680 nm, and the rate of ATP formation is 20 μ moles/cm²-hr. (a) What is the minimum flux in photons/cm²-sec at 680 nm? (b) What is the energy flux in cal/cm²-min and μ watts/cm² under the conditions of (a)? (c) If light of 430 nm is used, the ground state of chlorophyll, $S_{(\pi, \pi)}$, is excited to $S^b_{(\pi, \pi^*)}$. Suppose that 95% of $S^b_{(\pi, \pi^*)}$ goes to $S^a_{(\pi, \pi^*)}$ in 10^{-12} sec and that the rest of the upper excited singlet state returns to the ground state. What is the efficiency in terms of energy conversion of 430 nm light as an energy source for ATP formation compared with 680 nm light? (d) The

hydrolysis of 1 mole of ATP to ADP and phosphate under physiological conditions can yield about 10 kcal of free energy. What wavelength of light has the same amount of energy/"mole"?

4.4 Assume that some excited singlet state can become de-excited by three competing processes: (1) fluorescence (lifetime = 10^{-8} sec), (2) a radiationless transition to an excited triplet state (5×10^{-9} sec), and (3) a radiationless transition to the ground state (10^{-8} sec). (a) What is the lifetime of the excited singlet state? (b) What is the maximum quantum yield for de-excitations leading to electromagnetic radiation? (c) Suppose that the above molecule is inserted into a membrane, which adds a de-excitation pathway involving intermolecular transfer of energy from the excited singlet state (rate constant = 10^{12} sec $^{-1}$). What is the new lifetime of the excited singlet state?

4.5 The *cis*-isomer of some species has an absorption coefficient of 2.0×10^4 liters/mole-cm at 450 nm, where the spectrophotometer has a photon flux of 10^{13} photons/cm ^{2} -sec. (a) What concentration of the *cis*-isomer will absorb 65% of the incident 450 nm light for a cuvette (a transparent vessel used in a spectrophotometer) with an optical path length of 1 cm? (b) What is the absorbance of the solution in (a)? What would be the absorbance if the flux at 450 nm were decreased to 5×10^{12} photons/cm ^{2} -sec? (c) Suppose that the 450 nm light caused a photoisomerization of the *cis*-isomer to the *trans*-isomer with a quantum yield of 0.50, the other de-excitation pathway being the return to the *cis*-form. If the *trans*-isomer did not absorb at 450 nm, what would be the initial rate of decrease of the *cis*-isomer and rate of change of absorbance at 450 nm under the conditions of (a)? (d) If ϵ_{450} for the *trans*-isomer were 1.0×10^4 liters/mole-cm, and 450 nm light led to a photoisomerization of the *trans*-isomer with a quantum yield of 0.50 for forming the *cis*-isomer, what would be the ratio of *cis* to *trans* after a long time?

4.6 (a) Suppose that the spacing in wave numbers (see Problem 4.2) between vibrational sublevels for the transition depicted in Figure 4.6 is 1200 cm $^{-1}$ and that the most probable absorption predicted by the Franck-Condon principle occurs at 500 nm (the main band). What are the wavelength positions of the satellite bands which occur for transitions to the vibrational sublevels just above and just below the one for the most probable transition? (b) Suppose that the main band has a maximum molar absorption coefficient of 5×10^4 liters/mole-cm, while each satellite band has an ϵ_λ one-fifth as large. If 20% of the incident light is absorbed at the wavelengths of either of the satellite bands, what percentage is absorbed at the main band wavelength? (c) When the pigment is placed in a cuvette with an optical path length of 0.5 cm, the maximum absorbance is 0.3. What is the concentration?

4.7 A straight chain hydrocarbon has 11 double bonds in conjugation. Suppose that it has three bands in the visible region, one at 450 nm ($\epsilon_{450} = 1.0 \times 10^5$ liters/mole-cm), one at 431 nm ($\epsilon_{430} = 2 \times 10^4$ liters/mole-cm), and a minor band near 470 nm ($\epsilon_{470} \cong 1.0 \times 10^3$ liters/mole-cm). Upon cooling from 20° to liquid helium temperatures, the minor band essentially disappears. (a) What is the

splitting between vibrational sublevels? (b) What transition could account for the minor band? Support your answer by calculation. (c) If the $\lambda_{\max}$ for fluorescence is at 493 nm, what transition is responsible for the 450 nm absorption band? (d) If the double bond in the middle of the conjugated system is reduced (by adding 2 H's so that it becomes a single bond) and the rest of the molecule remains unchanged, calculate the new $\lambda_{\max}$ for the main absorption band and its absorption coefficient. Assume that for every double bond added to the conjugated system, $\lambda_{\max}$ shifts by 0.25 eV and $\epsilon_{\lambda_{\max}}$ is directly proportional to the number of double bonds in conjugation.

References

- Anderson, M. C. 1967. Photon flux, chlorophyll content, and photosynthesis under natural conditions. *Ecology* **48**: 1050-1053.
- Bickford, E. D., and S. Dunn. 1972. *Lighting for Plant Growth*, Kent State University Press, Kent (Ohio).
- Briggs, W. R., and H. V. Rice. 1972. Phytochrome: Chemical and physical properties and mechanism of action. *Annual Review of Plant Physiology* **23**: 293-334.
- Calvert, J. G., and J. N. Pitts, Jr. 1966. *Photochemistry*, Wiley, New York.
- Clayton, R. K. 1965. *Molecular Physics in Photosynthesis*, Blaisdell, New York.
- Clayton, R. K. 1970. *Light and Living Matter*, Vol. 1: *The Physical Part*, McGraw-Hill, New York.
- Depuy, C. H., and O. L. Chapman. 1972. *Molecular Reactions and Photochemistry*, Prentice-Hall, Englewood Cliffs (N.J.).
- Dyer, J. R. 1965. *Applications of Absorption Spectroscopy of Organic Compounds*, Prentice-Hall, Englewood Cliffs (N.J.).
- Halliday, D., and R. Resnick. 1960. *Physics for Students of Science and Engineering*, Wiley, New York.
- Jaffé, H. H., and M. Orchin. 1962. *Theory and Applications of Ultraviolet Spectroscopy*, Wiley, New York.
- Kaufman, J. E. (ed.). 1966. *IES Lighting Handbook*, Illuminating Engineering Society, New York.
- Levine, J. N. 1970. *Quantum Chemistry*, Vol. I: *Quantum Mechanics and Molecular Electronic Structure*, Allyn and Bacon, Boston.
- Mitrakos, K., and W. Shropshire, Jr. 1972. *Phytochrome*, Academic Press, New York.
- Mohr, H. 1972. *Photomorphogenesis*, Springer Verlag, New York.
- Monk, G. S. 1963. *Light: Principles and Experiments*, 2nd ed., Dover, New York.
- Moon, P. 1940. Proposed standard solar-radiation curves for engineering use. *Journal of the Franklin Institute* **230**: 583-617.
- Moore, W. J. 1972. *Physical Chemistry*, 4th ed., Prentice-Hall, Englewood Cliffs (N.J.).
- Munn, R. L. 1966. *Descriptive Meteorology*, Academic Press, New York.

- Orchin, M., and H. H. Jaffé. 1971. *Symmetry, Orbitals, and Spectra*, Interscience (Wiley), New York.
- Pauling, L. 1964. *College Chemistry*, 3rd ed., W. H. Freeman and Company, San Francisco.
- Salisbury, F. B., and C. Ross. 1969. *Plant Physiology*, Wadsworth, Belmont (Calif.).
- Sandorfy, C. 1964. *Electronic Spectra and Quantum Chemistry*, Prentice-Hall, Englewood Cliffs (N.J.).
- Scientific American*. 1968. Vol. 219, No. 3 (September), Special issue: Light.
- Seliger, H. H., and W. D. McElroy. 1965. *Light: Physical and Biological Action*, Academic Press, New York.
- Turro, N. J. 1967. *Molecular Photochemistry*, Benjamin, New York.
- van Holde, K. E. 1971. *Physical Biochemistry*, Prentice-Hall, Englewood Cliffs (N.J.).
- van Wijk, W. R. (ed.). 1966. *Physics of Plant Environment*, North-Holland, Amsterdam.
- Wald, G. 1968. Molecular basis of visual excitation. *Science* **162**: 230-239.

PHOTOSYNTHESIS

Photosynthesis is absolutely necessary for life as we know it. It is the largest scale synthetic process on earth. About 5×10^{16} g (50 billion tons) of carbon are fixed into organic compounds in photosynthetic organisms per year (we will consider this again at the end of the next chapter). Such a large number is difficult to visualize—it corresponds to an amount of organic compounds with a base of 1 km² and a height of over 100 km! The carbon source used in photosynthesis is the 0.03% CO₂ contained in the air and the CO₂ or HCO₃⁻ dissolved in lakes and oceans. In addition to the organic compounds, another product of photosynthesis essential for all respiring organisms is oxygen. At the present rate, the entire atmospheric content of oxygen is evolved by photosynthesis every few thousand years.

Photosynthesis is not a single reaction; it is composed of a great number of individual steps that work together at a remarkably high overall efficiency. We can divide the process into three stages: (1) the photochemical steps, our primary concern in this chapter (2) electron transfer, to which is coupled the formation of ATP (which we will consider in both this and the next chapter) and (3) the biochemical reactions involving the incorporation of CO₂ into carbohydrates. Figure 5.1 summarizes the processes involved and introduces the relative amounts of the various reactants and products taking part in the three stages of photosynthesis. The photochemical reactions, which are often referred to as the *primary events* of photosynthesis, lead to electron transfer along a sequence of molecules, which results in the formation of NADPH₂ and ATP.

Let us now consider the net chemical reaction for photosynthesis. In Figure 5.1, two waters are indicated as reactants in the oxygen evolution step and one water is a product in the biochemical stage. Hence, the overall *net* chemical reaction describing photosynthesis is carbon dioxide plus water

of 83 kcal/mole, so that $\frac{1}{2} \times 83$ has been indicated in the appropriate places in Eq. 5.1.) The total bond energy of the reactants in Equation 5.1 is 606 kcal/mole ($111 + 111 + 192 + 192 = 606$), and 498 kcal/mole for the products ($99 + 83 + 86 + 111 + 119 = 498$). The reactants H_2O and CO_2 represent the lower energy, since 108 kcal of energy/mole is necessary for the bond changes to convert them to the products $\{\text{CH}_2\text{O}\}$ plus O_2 . This energy change actually represents the increase in *enthalpy* required, ΔH , while we are really more concerned here with the change in Gibbs free energy, ΔG (see Ch. 6 and App. VII). (For a reaction at constant temperature, ΔG equals $\Delta H - T\Delta S$, where S is the entropy. ΔG is about the same as ΔH for Eq. 5.1.) Although the actual ΔG /mole of C depends somewhat on the particular carbohydrate involved, 114 kcal is approximately the increase in Gibbs free energy per mole of CO_2 which reacts in Equation 5.1. For instance, the Gibbs free energy released when glucose is oxidized to CO_2 and H_2O is 686 kcal/mole of glucose or 114 kcal/mole of C. In discussing photosynthesis we will frequently use this ΔG , which actually refers to standard state conditions (25° , pH 7, 1 molal concentrations, 1 atmosphere pressures).

About eight photons are required in photosynthesis per molecule of CO_2 fixed and O_2 evolved (cf. Fig. 5.1). Red light at 680 nm corresponds to 42.1 kcal/mole (Table 4.1), so that eight moles of such photons have (8) (42.1), or 337 kcal of radiant energy. Using this as the energy input, and 114 kcal/mole of CO_2 fixed as the amount of energy stored, the efficiency of energy conversion by photosynthesis is $(114/337)(100)$, or 34%. Actually, slightly more than 8 photons may be required per CO_2 fixed. Furthermore, the energy for wavelengths less than 680 nm, which are also used in photosynthesis, is higher than 42.1 kcal/mole. Both of these considerations lead to a calculated maximum efficiency of the utilization of absorbed energy of somewhat less than 34%. Nevertheless, photosynthesis is an extremely efficient energy conversion process, considering all the steps involved, each with its inherent energy losses.

Nearly all enzymes involved in the synthetic reactions of photosynthesis are also found in nonphotosynthetic tissue. Thus, the most unique feature of photosynthesis is the conversion of radiant energy into chemical energy. The present chapter will emphasize the light absorption and excitation transfer aspects of photosynthesis. We will consider the structures and absorption characteristics of photosynthetic pigments and the means by which radiant energy is trapped, transferred, and eventually used. Thus, the emphasis will be on the "photo" part of photosynthesis. In the next chapter, we will discuss energy conversion in a broader context, paying particular attention to ATP and NADPH_2 .

Chlorophyll—chemistry and spectra

The chlorophylls represent the principal class of pigments responsible for light absorption in photosynthesis and are found in all photosynthetic organisms. There are a number of different types of chlorophyll, as M. Tswett demonstrated in 1906 using adsorption chromatography. For instance, approximately 1 mg of the chlorophylls designated *a* and *b* is present per gram of fresh weight of green leaves. The empirical formulas were first given by R. Willstätter, beginning in 1913, and H. Fisher established the actual structures of various chlorophylls by 1940. These two investigators, as well as R. B. Woodward, who first synthesized chlorophyll *in vitro*, have all been recipients of the Nobel prize for their studies on this important plant pigment. We will consider the structure of chlorophyll *a*, then the all-important absorption properties of the chlorophylls and their fluorescence characteristics.

Types and structures. The various types of chlorophyll are identified by letters or by the taxonomic group of the plants in which they occur. One of the most important ones is chlorophyll *a*. It has a molecular weight of 893.5, and its structure is given in Figure 5.2. Chlorophyll *a* is found in all photosynthetic organisms except bacteria, i.e., in all species where oxygen evolution accompanies photosynthesis. It is a tetrapyrrole (cf. p. 205) having a relatively flat porphyrin "head" about 1.5 nm by 1.5 nm (15 Å by 15 Å), in the center of which a magnesium atom is covalently bound. Attached to the head is a long-chain terpene alcohol, phytol, which is like a "tail" about 2 nm (20 Å) in length and containing 20 carbon atoms (Fig. 5.2). This tail provides a nonpolar anchor which is probably important in situating the chlorophyll molecules in the lamellar membranes in the proper relation to each other and to the other components with which they interact. However, the phytol part makes no appreciable contribution to the optical properties of chlorophyll in the visible region. The system of rings in the porphyrin head of the chlorophyll *a* molecule is highly conjugated, having nine double bonds in conjugation (plus three other double bonds occurring in branches to the main conjugated system). These alternating single and double bonds of the porphyrin ring provide many delocalized π electrons which can take part in the absorption of light.

A number of other chlorophyll forms structurally similar to chlorophyll *a* occur in nature. For instance, chlorophyll *b* differs from chlorophyll *a* only by having a formyl group ($-\text{CHO}$) in place of a methyl group ($-\text{CH}_3$) on ring II. Chlorophyll *b* is found in most land plants (including ferns and mosses), the green algae, and the Euglenophyta; the ratio of chlorophyll *a* to chlorophyll *b* in these organisms is usually close to three. Chlorophyll

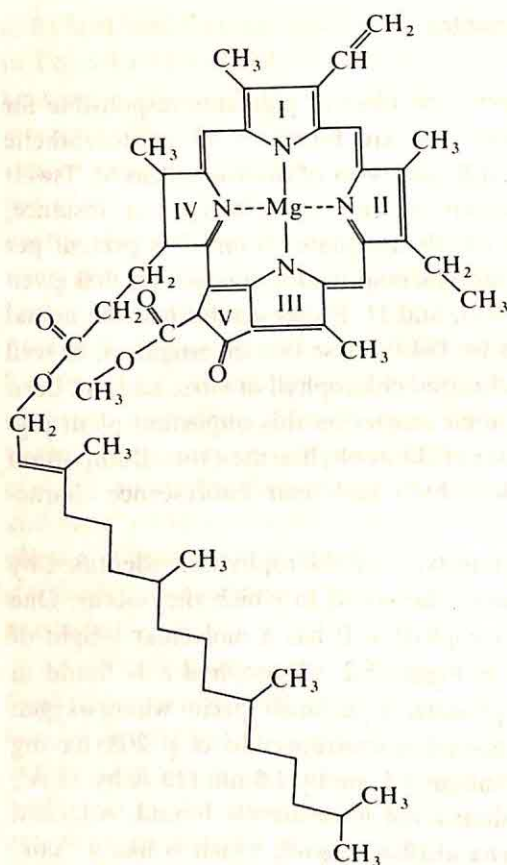


Figure 5.2

Structure of chlorophyll *a*, illustrating the highly conjugated porphyrin "head" to which is attached a phytol "tail." The convention for numbering the various rings is also indicated.

b is not essential for photosynthesis, since a barley mutant is known to contain only chlorophyll *a*, yet it carries out photosynthesis quite satisfactorily (see Levine).

Another type is chlorophyll *c*, which occurs in the dinoflagellates, cryptomonads, diatoms, golden algae, and the brown algae. The common chlorophyll of bacteria is called bacteriochlorophyll *a*. The purple photosynthetic bacteria contain bacteriochlorophyll *a* (or *b* in some species), while bacteriochlorophyll *a* plus one of two forms of chlorobium chlorophyll occur in

green photosynthetic bacteria. These bacterial pigments differ from green plant chlorophylls in that they contain two more hydrogens in the porphyrin ring. They also have different substituents around the periphery of the porphyrin ring; in addition, chlorobium chlorophyll has the alcohol farnesol (15 C and three double bonds) in the place of phytol (20 C and 1 double bond). For convenience, we will abbreviate chlorophyll *a*, which is the pigment of principal interest in this text, by Chl *a* (see Goodwin; Ó hEocha; Rabinowitch and Govindjee; or Vernon and Seely for further comments on the distribution of chlorophylls among various plants).

Absorption and fluorescence emission spectra. The absorption spectrum of Chl *a* has a blue and a red band, hence, the characteristic color of chlorophyll is green. The band in the blue part of the spectrum has a peak at 430 nm for Chl *a* in ether (Fig. 5.3). This band is known as the *Soret band* and occurs in the long-wavelength part of the UV, the violet, or the blue region for all tetrapyrroles. We will designate the wavelength position for a local maximum of the absorption coefficient in an absorption band by λ_{max} . The molar absorption coefficient at the λ_{max} for the Soret band of Chl *a* is just over 1.2×10^5 liters/mole-cm (Fig. 5.3). Such a high value is a consequence of the many double bonds in the conjugated system of

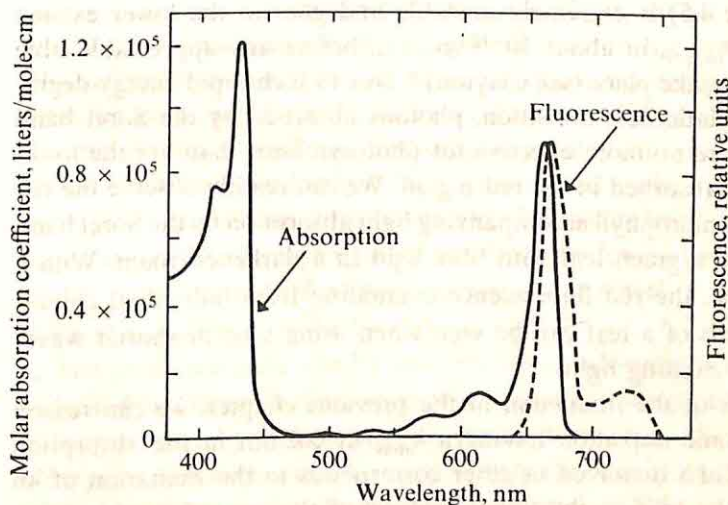


Figure 5.3

Absorption and fluorescence emission spectra of Chl *a* dissolved in ether. (Source: A. S. Holt and E. E. Jacobs, *American Journal of Botany* **41**: 710-717 (1954). Data reprinted by permission.)

the porphyrin ring of chlorophyll. Chl *a* has a major band in the red region, which has a $\lambda_{\max}$ at 662 nm when the pigment is dissolved in ether (Fig. 5.3). (See Clayton; Rabinowitch and Govindjee, or Vernon and Seely.)

Chl *a* also has a number of minor absorption bands. For instance, Chl *a* dissolved in ether has a small absorption band at 615 nm, which is located 47 nm to the short wavelength side of the main red band (Fig. 5.3). Absorption of light with a wavelength of 615 nm leads to an electronic transition requiring 0.14 eV more energy than the main band at 662 nm. This extra energy is similar to the energy spacing between vibrational sublevels. Therefore, this small band on the shorter wavelength (higher energy) side of the red band could correspond to electrons going to the vibrational sublevel in the excited state immediately above the sublevel for the $\lambda_{\max}$ at 662 nm—an aspect we will return to shortly. Also, there is a small band centered near 410 nm (Fig. 5.3) which represents a transition requiring 0.14 eV more energy than the main Soret band at 430 nm. This satellite band may represent a transition to the vibrational sublevel above that for the Soret band of Chl *a* (see Fig. 4.6 for an illustration of vibrational sublevels).

The fluorescence emission spectrum of Chl *a* in ether is also presented in Figure 5.3. Although chlorophyll absorbs strongly in both the red and the blue, the fluorescence is essentially all in the red region. This is due to the fact that the upper singlet state of chlorophyll excited by blue light ($S^b_{(\pi, \pi^*)}$ in Fig. 4.5) is extremely unstable and goes to the lower excited singlet state, $S^a_{(\pi, \pi^*)}$, in about 10^{-12} sec, i.e., before any appreciable blue fluorescence can take place (see Clayton).^{*} Due to such rapid energy degradation by a radiationless transition, photons absorbed by the Soret band of chlorophyll are no more effective for photosynthesis than are the lower energy photons absorbed in the red region. We can readily observe the red fluorescence of chlorophyll accompanying light absorption by the Soret band if we illuminate a green leaf with blue light in a darkened room. With a light microscope, the red fluorescence emanating from individual chloroplasts in the cells of a leaf can be seen when using blue or shorter wavelengths for the exciting light.

On the basis of the discussion in the previous chapter, we can reason that the electronic transition having a $\lambda_{\max}$ at 662 nm in the absorption spectrum for Chl *a* dissolved in ether corresponds to the excitation of an electron from the lowest vibrational sublevel of the ground state to some

^{*} $S^b_{(\pi, \pi^*)}$ and $S^a_{(\pi, \pi^*)}$ are distinct excited electronic states; each has its own energy curve in a diagram like Figure 4.6.

vibrational sublevel of the lower excited state. We can use the Boltzmann factor ($n(E)/n_{\text{total}} = e^{-E/kT}$, Eq. 3.21) to estimate the fraction of chlorophyll molecules in the first excited vibrational sublevel of the ground state at a typical plant temperature. Since kT is 0.0257 eV at 25° (App. III) and the distance between vibrational sublevels is about 0.14 eV for chlorophyll, the Boltzmann factor could equal $e^{-(0.14 \text{ eV})/(0.0257 \text{ eV})}$, or $e^{-5.4}$, which is 0.0045. That is, less than one in two hundred chlorophyll molecules would normally be in the first excited vibrational sublevel of the ground state when light arrives. Consequently, the absorption of a photon will most likely occur when chlorophyll is in the lowest vibrational sublevel of the ground state.

In Chapter 4 we argued that fluorescence generally occurs from the lowest vibrational sublevel of the excited singlet state. In other words, any excess vibrational energy is usually dissipated before the rest of the energy of the absorbed photon is reradiated as fluorescence. But Figure 5.3 clearly shows that the wavelength region for most of the fluorescence is at about the same location as the red band in the chlorophyll absorption spectrum. In particular, the λ_{max} for fluorescence occurs at 666 nm, which is only 0.01 eV lower in energy than the λ_{max} of 662 nm for the red band in the absorption spectrum. (The slight shift, which is much less than the distance between vibrational sublevels of 0.14 eV for chlorophyll, is most likely due to the loss of some rotational energy, e.g., rotational subsublevels of a vibrational sublevel are generally about 0.01 eV apart.) Thus, the transition from the lowest vibrational sublevel of the ground state to the lower excited state (the red absorption band) has essentially the same energy as a transition from the lowest vibrational sublevel of that excited state to the ground state (the red fluorescence band). The only way for this to occur is to have the lowest vibrational sublevels of both ground and excited states involved in each of the transitions. Hence, the red absorption band corresponds to a transition of the chlorophyll molecule from the lowest vibrational sublevel of the ground state to the lowest vibrational sublevel of the lower excited state, as is indicated in Figure 5.4.

The participation of the lowest vibrational sublevels of both the ground state and the lower excited state of Chl *a* in the main red band can also be appreciated by comparing the bands at 662 nm and 615 nm in the absorption spectrum with the two bands in the fluorescence emission spectrum. The shorter wavelength absorption band at 615 nm—0.14 eV higher in energy than the 662 nm band—corresponds to a transition to the first excited vibrational sublevel in the lower excited state. De-excitations from the lowest vibrational sublevel of the lower excited state to excited vibrational sublevels of the ground state would correspond to fluorescence at

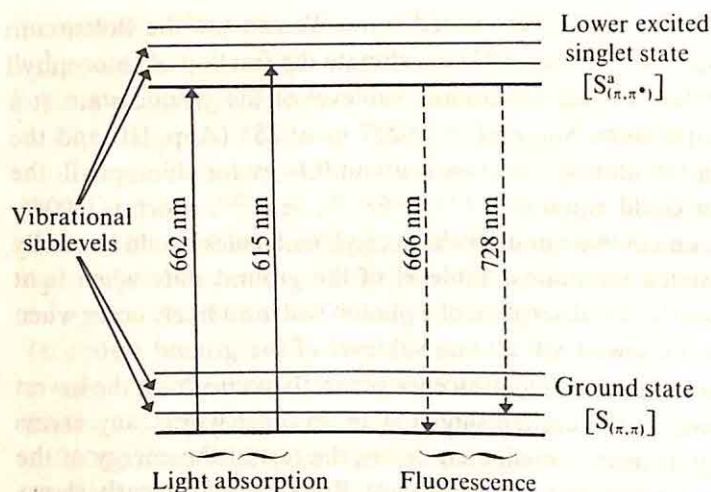


Figure 5.4

Energy level diagram, indicating the vibrational sublevels of the ground state $[S_{(\pi,\pi)}]$ and the lower excited singlet state $[S^a_{(\pi,\pi^*)}]$ of Chl *a* dissolved in ether; solid vertical lines indicate the absorption of light by Chl *a*; dashed lines represent fluorescence at the specified wavelengths. The lengths of the arrows are proportional to the amounts of energy involved in the various transitions.

wavelengths greater than 700 nm. In fact, a small band near 728 nm in the fluorescence emission spectrum of Chl *a* (Fig. 5.3) occurs about 60 nm on the long wavelength side of the main fluorescence band, indicating an electronic transition with 0.15 eV less energy than the 666 nm band. This far-red band most likely corresponds to fluorescence emitted as the chlorophyll molecule goes from the lowest vibrational sublevel of the lower excited state to the first excited vibrational sublevel of the ground state. Figure 5.4 summarizes the various vibrational sublevels and the electronic transitions we have discussed for light absorption and fluorescence emission by Chl *a* in ether.

Absorption bands *in vivo*. Chl *a* occurs in a number of differently absorbing forms *in vivo*. Each type is conveniently described by the $\lambda_{\max}$ of its red band. The form which has a peak near 670 nm will be referred to as Chl *a*₆₇₀. Another type of Chl *a* has a $\lambda_{\max}$ near 678 to 680 nm and we will designate it by Chl *a*₆₈₀. Although at least four major forms of Chl *a* occur *in vivo* (see Brown), we will restrict our attention to Chl *a*₆₇₀ and Chl *a*₆₈₀. A special Chl *a* (one in approximately 450 chlorophylls) has a $\lambda_{\max}$ near 700 nm and is termed P₇₀₀ (P indicating pigment). Even though

only a small fraction of Chl *a* occurs as P_{700} , it plays a very important role in photosynthesis.

The various values of $\lambda_{\max}$ for Chl *a* *in vivo* result from interactions between the chlorophylls and surrounding molecules, such as the proteins and lipids in the lamellae as well as adjacent water molecules. Chl *a* is actually bound in a special chlorophyll-protein complex, most likely by means of hydrophobic interactions between the protein and the phytol tail of chlorophyll. Because of the interactions of the porphyrin ring with the other molecules in the complex, and especially with the polar amino acids of the protein, the red bands for Chl *a* *in vivo* are shifted toward longer wavelengths than for Chl *a* dissolved in ether ($\lambda_{\max}$ at 662 nm). This is an example of the pronounced effect that the solvent or other neighboring molecules can have in determining the positions of the electronic energy levels of a pigment. P_{700} is apparently located in an environment where neighboring molecules strongly interact with it; this markedly influences the electron distribution within P_{700} , and consequently alters its absorption properties compared with other Chl *a*'s. The red absorption band of Chl *b* *in vivo* occurs as a shoulder on the low wavelength side of the Chl *a* red band, usually near 650 nm.

We can describe the bandwidth of an absorption band by the difference in energy between photons on the two sides of the band at wavelengths where the absorption has dropped to half of that for $\lambda_{\max}$. C. S. French has found that such bandwidths of the red absorption bands of the various Chl *a*'s *in vivo* are actually fairly narrow—often about 10 nm at 20° (see Brown). At 680 nm a bandwidth of 10 nm is equivalent to 0.03 ev in energy, i.e., a photon having a wavelength of 675 nm has an energy which is 0.03 ev greater than a photon with a wavelength 10 nm longer (685 nm). An energy of 0.03 ev is somewhat smaller than the usual energy difference between vibrational sublevels—the spacing of vibrational sublevels is about 0.14 ev for Chl *a*, as we indicated above. Thus, a bandwidth of 0.03 ev results mainly from the rotational and the translational broadening of an electronic transition to a single vibrational sublevel of the excited state of Chl *a*.

The absorption of polarized incident light by chlorophyll *in vivo* can provide information on the orientations between individual chlorophyll molecules. ("Polarized" simply means that the oscillating electric vector of light, Fig. 4.1, is in some specified direction.) The electronic transition of chlorophyll to the excited singlet state responsible for the red absorption band has its electric dipole in the plane of the porphyrin ring. Polarized light of the appropriate wavelength which has its oscillating electric vector parallel to this plane is therefore preferentially absorbed by chloro-

phyll—recall that the probability for absorption is proportional to the square of the cosine of the angle between the induced dipole and the electric field vector of light (cf. p. 176). Using polarized light, we can show that the porphyrin rings of a few percent of the Chl *a* molecules, perhaps including P₇₀₀, are nearly parallel to the plane of the chloroplast lamellae (see Vernon and Seely). However, most of the chlorophyll molecules have their porphyrin heads oriented in a random fashion in the internal membranes of the chloroplasts.

Observations on the degree of polarization of fluorescence following the absorption of polarized light can tell us whether the excitation has been transferred from one molecule to another. If the same chlorophyll molecules which absorbed polarized light later emit photons when they go back to the ground state, the fluorescence would be polarized to within a few degrees of the direction of the electric vector of the incident light. However, the chlorophyll fluorescence following absorption of polarized light by chloroplasts is not appreciably polarized (see Clayton or Goedheer). We could explain this *fluorescence depolarization* if the excitation energy had been transferred from one chlorophyll molecule to another so many times that the directional aspect had become randomized, i.e., if the fluorescence emitter were randomly aligned relative to the chlorophyll molecule which absorbed the polarized light. Such fluorescence depolarization has important implications for the excitation transfer reactions of chlorophyll which we will discuss in a later section.

Other photosynthetic pigments

Besides chlorophyll, other molecules in photosynthetic organisms also absorb light in the visible region. If these molecules pass their electronic excitations on to Chl *a*, they are known as auxiliary or “accessory” pigments. In addition to chlorophylls *b* and *c*, two groups of accessory pigments important to photosynthesis are the carotenoids and the phycobilins. These two classes of accessory pigments can absorb yellow or green light, wavelengths where absorption by chlorophyll is not appreciable.

Fluorescence studies have provided valuable information on the sequence of excitation transfer to and from the accessory pigments. For example, light absorbed by carotenoids, phycobilins, and Chl *b* leads to the fluorescence of Chl *a*. However, light absorbed by Chl *a* does not lead to the fluorescence of any of the accessory pigments, suggesting that excitation energy is not transferred from Chl *a* to the accessory pigments (see Goedheer, or Rabino-

witch and Govindjee). Thus, accessory pigments can increase the photosynthetic use of white light by absorbing at wavelengths where chlorophyll absorption is low; the excitation is then transferred to Chl *a* before the photochemical reactions take place.

Carotenoids. Carotenoids are found in essentially all green plants, algae, and photosynthetic bacteria (see Goodwin or Isler). In fact, the dominant pigments for plant leaves are the chlorophylls, which absorb strongly in the red and the blue regions, and the carotenoids, which absorb mostly in the blue and somewhat in the green region of the spectrum. The predominant colors reflected or transmitted by leaves are therefore green and yellow. In the fall season, chlorophyll in the leaves of deciduous plants can bleach and not be replaced, greatly reducing absorption in the red and the blue regions. The remaining carotenoids absorb only in the blue and green regions of the visible part of the spectrum, leading to the well-known colors of such leaves in the fall, viz., yellow, orange, and red. Animals do not synthesize carotenoids. Brightly colored birds such as canaries and flamingoes, as well as many invertebrates, obtain their yellow or reddish colors from the carotenoids in the parts of the plants which they eat.

The chlorophylls and the carotenoids are embedded in the chloroplast lamellar membranes, which we discussed in Chapter 1 (cf. Fig. 1.9). When viewed with an electron microscope, these lamellae appear to have oblate spheroids about 10 nm by 17 nm as part of their substructure. These were named *quantasomes* by R. Park, and were shown to contain approximately 230 chlorophylls and 50 carotenoids, which gives an indication of the relative frequency of these two pigments in photosynthesis in higher plants (see Park and Biggins). Carotenoids also occur in organelles known as *chromoplasts*, which are about the size of chloroplasts and are often derived from them. Lycopene (red) is in tomato chromoplasts, α - and β -carotenes (orange) occur in carrot root chromoplasts, while the various chromoplasts of flowers contain a great diversity of carotenoids (see Kirk and Tilney-Bassett for further details on chromoplasts).

Carotenoids are 40-carbon terpenoids or isoprenoids. They are composed of 8 isoprene units, where isoprene is a 5-carbon compound having two double bonds ($\text{CH}_2=\text{CCH}=\text{CH}_2$).



units on one or both ends of the molecule are part of six-membered rings (see Fig. 5.6). Carotenoids are about 3 nm (30 Å) long, and those involved in photosynthesis generally contain 9 or more double bonds in conjugation.

The wavelength position of the $\lambda_{\max}$ depends on the solvent, on the substitutions on the hydrocarbon backbone, and on the number of double bonds in the conjugated system. We can illustrate this latter point for carotenoids in *n*-hexane, where the central maxima of the three observed peaks in the absorption spectra are at 285 nm for 3 double bonds in conjugation, at 348 nm for 5, at 400 nm for 7, at 440 nm for 9, and at 472 nm for 11 double bonds in conjugation—the only change from molecule to molecule in this series is in the number of double bonds in conjugation (see Goodwin). Thus, the greater the degree of conjugation, the longer is the wavelength representing $\lambda_{\max}$, as we discussed in Chapter 4. For the 9 to 12 double bonds occurring in the conjugated systems of photosynthetically important carotenoids, the maximum absorption coefficient is greater than 10^5 liters/mole-cm.

The carotenoids which serve as accessory pigments for photosynthesis absorb strongly in the blue (425 to 490 nm, Table 4.1) and somewhat in the green (490 to 560 nm), usually having triple-banded spectra in the region from 400 to 540 nm. For β -carotene in hexane, the three bands are centered at 425, 451, and 483 nm (Fig. 5.5), while another major carotenoid, lutein, has peaks at 420, 447, and 477 nm when dissolved in ethanol (its absorption

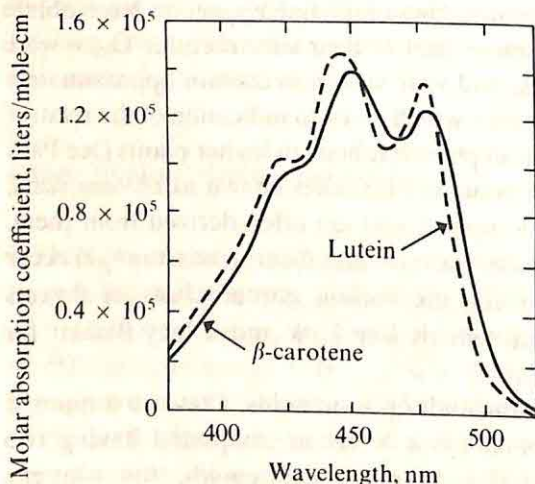


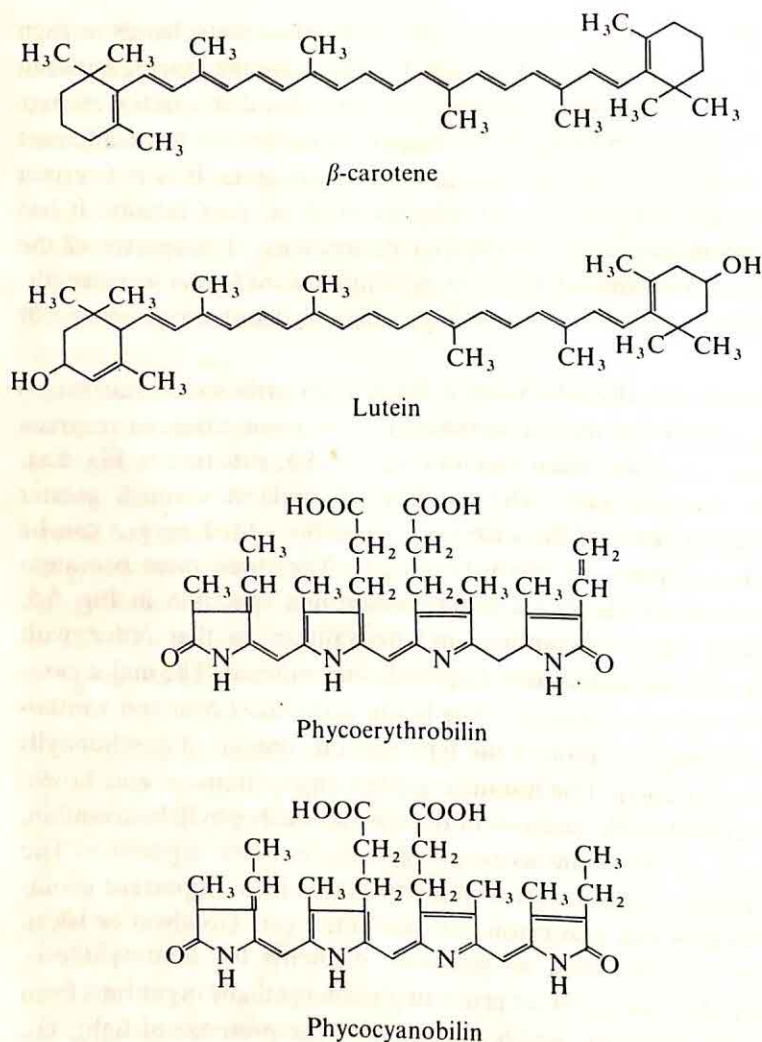
Figure 5.5

Absorption spectra for the two major carotenoids of green plants. (Data for β -carotene (in *n*-hexane) and lutein (in ethanol) are from F. P. Zscheile, J. W. White, Jr., B. W. Beadle, and J. R. Roach, *Plant Physiology* 17: 331–346 (1942). Used by permission.)

spectrum is also indicated in Fig. 5.5). The three absorption bands in each spectrum are 0.17 to 0.18 eV apart, reasonable values for the spacing between adjacent vibrational sublevels. Hence, the triple-banded spectra characteristic of carotenoids most likely represent transitions to three adjacent vibrational sublevels in the same excited electronic state. It is not certain which vibrational sublevels are actually involved, in part because it has proved difficult to detect any carotenoid fluorescence. The spectra of the carotenoids *in vivo* are shifted about 20 to 30 nm toward longer wavelengths compared with absorption when the pigments are dissolved in hexane or ethanol.

Carotenoids are usually subdivided into the hydrocarbon ones (carotenes) and the oxygenated species (xanthophylls). The major carotene in green plants is β -carotene (absorption spectrum in Fig. 5.5, structure in Fig. 5.6); α -carotene is also abundant. The xanthophylls exhibit a much greater structural diversity than do the carotenes, since the added oxygen can be in hydroxy, keto, epoxy, or methoxy groups. The three most prevalent xanthophylls in green plants are lutein (absorption spectrum in Fig. 5.5, structure in Fig. 5.6), violaxanthin, and neoxanthin, in that order, with cryptoxanthin and zeaxanthin less frequently encountered. The major carotene of algae is again β -carotene, while lutein is the most common xanthophyll, although great variation in the type and the amount of xanthophylls is characteristic of algae. For instance, golden algae, diatoms, and brown algae contain considerable amounts of the special xanthophyll, fucoxanthin, which functions as the main accessory pigment in these organisms. The distribution and the types of carotenoids in plants have important evolutionary implications and also taxonomic usefulness (see Goodwin or Isler).

In addition to functioning as accessory pigments for photosynthesis, carotenoids are also important for protecting photosynthetic organisms from destructive photooxidations which can occur in the presence of light, O_2 , and certain pigments. In particular, light absorbed by chlorophyll can lead to excited states of molecular oxygen. These highly reactive states of O_2 can damage chlorophyll, but their interactions with carotenoids prevent harmful effects to the organism. For instance, a mutant of *Rhodospirillum rubrum* lacking carotenoids performs photosynthesis in a normal manner in the absence of oxygen; when O_2 is introduced in the light, the bacteriochlorophyll becomes photooxidized and the bacteria are killed, a sensitivity not occurring in related strains containing carotenoids. In other cases, when carotenoid synthesis is inhibited, light in the presence of oxygen can be lethal to the photosynthetic organism. Finally, since bacterial photosynthesis does not lead to oxygen evolution, it can proceed in the absence of carotenoids. However, algae and higher plants produce O_2 as a photo-

**Figure 5.6**

Structure of four important accessory pigments.

synthetic product, and thus they must contain carotenoids in order to survive in the light (see Fogg and Isler).

Phycobilins. The other main accessory pigments important in photosynthesis are the phycobilins. R. Lemberg in the 1920s termed these molecules *phycobilins* because they resembled bile pigments. They occur associated with the surfaces of the lamellar membranes in blue-green and red algae as well as in the cryptomonads (a small class containing flagellated unicellular algae).

Like the chlorophylls, phycobilins are tetrapyrroles. However, the four pyrroles in the phycobilins occur in an open chain, not in a closed porphyrin ring, as is the case for the chlorophylls. Phycobilins, which have molecular weights close to 586, occur covalently bound to proteins that generally have molecular weights of 30,000 to 40,000. Three pigment molecules are often attached to the same protein. *In vivo* the proteins can occur in aggregates having molecular weights from about 150,000 to over 300,000 (see Glazer *et al.*; Goodwin; Ó hEocha; or Rosowski and Parker for further details on phycobilins).

Phycobilins generally have their major absorption bands in the region from 500 to 650 nm, with the Soret band occurring in the UV (Fig. 5.7). These pigments are higher in concentration in many algae than are the chlorophylls and are responsible for the color of certain species. The main phycobilins are phycocyanobilin and phycoerythrobilin (structures given in Fig. 5.6). Phycoerythrobilin plus the protein to which it is attached is called *phycoerythrin*. Phycoerythrin is soluble in aqueous solutions, and thus we can obtain absorption spectra for it under conditions similar to those *in vivo*. Phycoerythrin is reddish, since it absorbs green and has at least one main band between 530 and 570 nm (see absorption spectrum in Fig. 5.7). It occurs

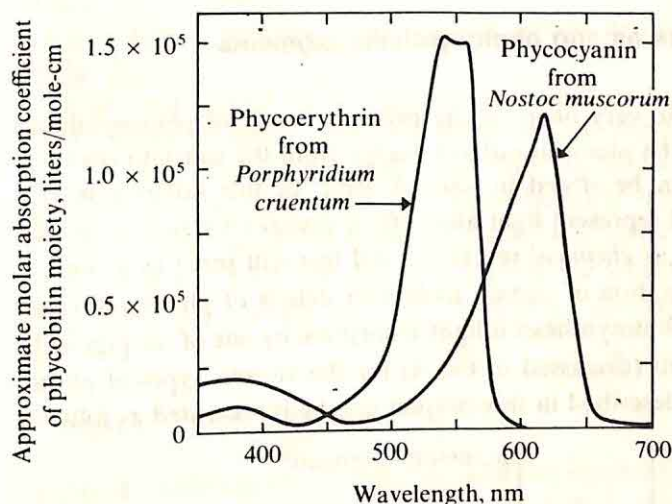


Figure 5.7

Absorption spectra of phycoerythrin from a red alga and phycocyanin from a blue-green alga. (Data are replotted from C. Ó hEocha, in *Chemistry and Biochemistry of Plant Pigments*, T. W. Goodwin, ed., Academic Press, London, 1965, pp. 175–196. Used by permission.)

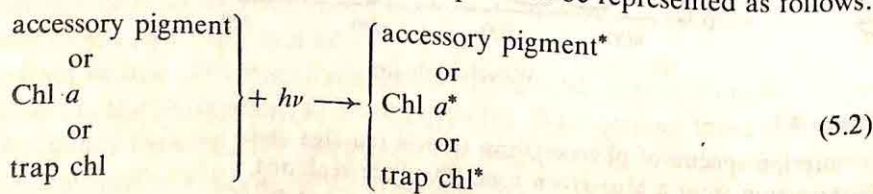
throughout the red algae, in some blue-green algae, and in the cryptomonads. *Phycocyanin* (phycocyanobilin plus protein) appears bluish, because it absorbs strongly from 610 to 660 nm (Fig. 5.7). Phycocyanin is the main phycobilin in the blue-green algae and is also found in the red algae and the cryptomonads. As is the case for other pigments, the greater the number of double bonds in conjugation in the phycobilins, the longer are the wavelengths for $\lambda_{\max}$. For example, phycoerythrobilin has 6 double bonds in the main conjugated system and absorbs maximally in the green region of the spectrum, while phycocyanobilin has 9 such double bonds and its $\lambda_{\max}$ occurs in the red (cf. the structures for these compounds in Fig. 5.6). As indicated in Figure 5.7, the maximum absorption coefficients of both phycobilins exceed 10^5 liters/mole-cm.

Interestingly enough, only two types of pigments appear to be involved in all the known photochemical reactions in plants. These are the carotenoids and the tetrapyrroles, the latter class including the chlorophylls, the phycobilins, and phytochrome. The maximum absorption coefficients for the most intense absorption bands are slightly over 10^5 liters/mole-cm in each case. Cytochromes, which are involved in the electron transport reactions in chloroplasts and mitochondria, are also tetrapyrroles, as we will indicate later in this chapter.

Excitation transfers among photosynthetic pigments

Chlorophyll is at the very heart of the primary events of photosynthesis. It helps to convert the plentiful radiant energy from the sun into chemical free energy that can be stored in various ways. In this rather extended introduction we will represent light absorption, excitation transfer, and the photochemical step as *chemical* reactions, and this will serve as a prelude to a further consideration of certain molecular details of photosynthesis.

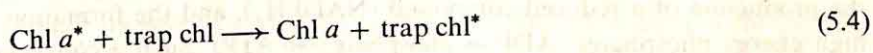
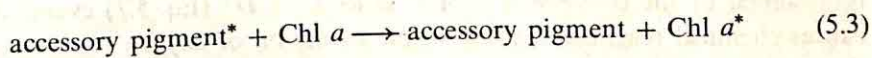
The first step in photosynthesis is light absorption by one of the pigments. The absorption event (discussed in Ch. 4) for the various types of photosynthetic pigments described in this chapter can be represented as follows:



where the asterisk refers to an excited state of the pigment molecule caused

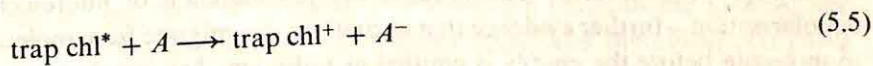
by the absorption of a light quantum, $h\nu$. "Trap chl" indicates a special type of Chl a (e.g., P_{700}) which occurs rather infrequently; we will consider its important excitation-trapping properties at the end of this section.

Since the photochemical reactions take place only at the trap Chl molecules, the excitations resulting from light absorption by either the accessory pigments or the regular Chl a 's must migrate to the trap chl before they can be used for photosynthesis. Trap chl is relatively rare compared with the other photosynthetic pigments and therefore absorbs only a small fraction of the incident light. In fact, under natural conditions in green plants over 99% of the photons are absorbed by either the accessory pigments or Chl a . The migration of excitations from the initially excited species to the trap chl—the mechanism of which we will discuss below—can be represented as follows:



In other words, the direction of excitation transfer or migration is from the accessory pigments to Chl a (Eq. 5.3) and from Chl a to the special "trap" chlorophylls (Eq. 5.4) where the actual photochemical reactions take place. Hence, the overall effect of the steps described by Equations 5.2 through 5.4 is to funnel the excitations that are caused by the absorption of light to the trap chl.

A prerequisite for the conversion of radiant energy into a form which can be stored chemically is the formation of reducing and oxidizing species. The reducing (electron-donating) and oxidizing (electron-accepting) species which result from light absorption must be fairly stable and separated in such a way that they do not interact. (We will discuss oxidation and reduction in detail in Ch. 6.) If we denote the molecule which accepts an electron from the excited trap chl by A , this electron transfer step can be represented by



where A^- indicates the reduced state of the acceptor and trap chl^+ means that the special chlorophyll has lost an electron. Equation 5.5 represents a *photochemical* reaction since the absorption of a light quantum (Eq. 5.2) has led to the transfer of an electron away from a special type of chlorophyll, representing a chemical change in that molecule. The electron removed from trap chl^* (Eq. 5.5) can be replaced by one coming from a donor, D , which

leads to the oxidation of this latter species (D^+) and the return of the trap chl to its normal unexcited state:



The generation of stable reduced (A^-) and oxidized (D^+) intermediates can be considered to complete the conversion of light energy into chemical potential energy. In this connection, it is convenient to combine Equations 5.2 through 5.6, which gives us the following relation for the net reaction describing the primary events of photosynthesis:



In green plants and algae the light-driven change in chemical free energy represented by the conversion of $A + D$ to $A^- + D^+$ (Eq. 5.7) eventually causes chemical reactions leading to the evolution of oxygen from water, the production of a reduced compound (NADPH_2), and the formation of high energy phosphates ($\text{ADP} + \text{phosphate} \longrightarrow \text{ATP}$). Such a conversion of light energy into chemical energy represented by Equation 5.7 is the cornerstone of photosynthesis.

In this section we will consider a mechanism for the transfer of excitation from an excited molecule to another in its ground state. The concepts developed will then be applied to the specific case of the migration of an excitation among photosynthetic pigments, i.e., reactions described by Equations 5.3 and 5.4. Finally, we will discuss the actual trapping of the excitation in special Chl *a* molecules. This trapping is followed by the separation of an electron from chlorophyll, a necessary step for the generation of chemical potential energy.

Resonance transfer of excitation. We have already mentioned a number of different examples of excitation transfer between photosynthetic pigments. For instance, light absorbed by the accessory pigments can lead to the fluorescence of Chl *a*. Also, studies on the absorption of polarized light by chlorophyll *in vivo* have demonstrated the phenomenon of fluorescence depolarization—further evidence that excitations can migrate from molecule to molecule before the energy is emitted as radiation. The simplest case to consider is the transfer of excitation between identical molecules, e.g., the excitation of the lower excited singlet state of chlorophyll can be passed on to a second chlorophyll molecule. This causes the deactivation of the originally excited molecule and the attainment of the lower excited singlet state in the second chlorophyll, a process described by Equation 4.6, $S_{(\pi, \pi^*)} + S_{2(\pi, \pi)} \longrightarrow S_{(\pi, \pi)} + S_{2(\pi, \pi^*)}$. The most widely accepted mechanism

for such exchange of electronic excitation between chlorophyll molecules is "resonance transfer," which we will next consider qualitatively.

On the basis of our discussion in the previous chapter, we might expect that an excited molecule could induce an excited state in a second molecule in close proximity. The oscillating electric dipole representing the energetic electron in the excited state of the first molecule leads to a varying electric field. This field can cause a similar oscillation or resonance of some electron in a second molecule. A transfer of electronic excitation energy takes place when the electron oscillation in the second molecule is induced. When excitation transfer is completed, the previously excited electron in the first molecule has ceased oscillating while some electron in the second molecule is now oscillating, leading to an excited state of that molecule. Resonance transfer of excitation between molecules is thus analogous to the process whereby light is originally absorbed, since an oscillation of some electron in the molecule is induced by a locally varying electric field (see p. 176). Resonance transfer of excitation is most probable when there is the proper orientation between the electric dipole in the excited molecule and the potential dipole in the second molecule *and* the energy of the original dipole is appropriate, an aspect we will consider next.

In order for resonance transfer of electronic excitation to occur, the energy available in the excited molecule must match the energy that can be accepted by a second molecule. The wavelengths for fluorescence indicate the energy in the excited singlet state of a molecule (at least after the very rapid radiationless transitions to the lowest vibrational sublevel of that excited state have occurred). Thus, the fluorescence emission spectrum usually gives the range of energies available for transfer to a second molecule. On the other hand, the range of wavelengths of light which can sympathetically induce an oscillation of some electron in a second molecule is given by the absorption spectrum of that molecule (Ch. 4), and therefore the absorption spectrum shows the energies which can be accepted by a molecule. As might be expected from these two considerations, the probability for resonance transfer is high when the overlap in wavelength between the fluorescence band for the excited oscillator (available energy) and the absorption band of an unexcited oscillator (acceptable energy) in a neighboring molecule is large. The absorption and the fluorescence spectra of Chl *a* are both given in Figure 5.3. Since the overlap in the red region of the two spectra is very great, excitations can be efficiently exchanged between Chl *a* molecules by resonance transfer. Figure 5.8 illustrates the various energy considerations involved in the case of resonance transfer of excitation between two different types of molecules.

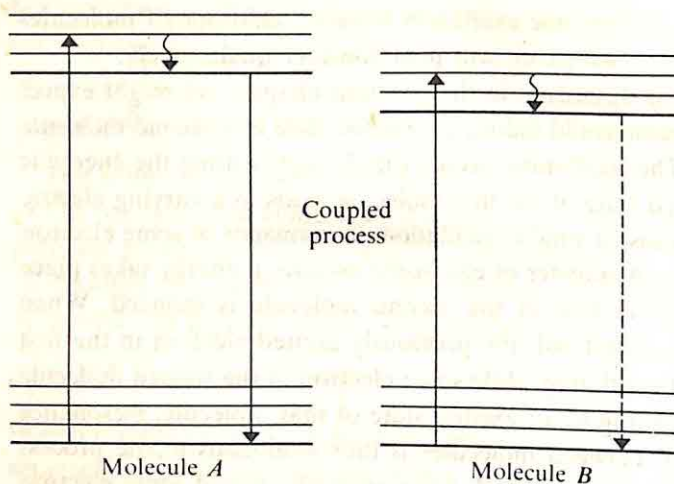


Figure 5.8

Resonance transfer of excitation from molecule *A* to molecule *B*. After light absorption by molecule *A*, there is a radiationless transition to the lowest vibrational sublevel of its excited state. Next, resonance transfer of the excitation takes place from *A* to *B*, causing the second molecule to go to an excited state, while molecule *A* returns to its ground state. Following a radiationless transition to the lowest vibrational sublevel in the excited state, fluorescence is then emitted by molecule *B* as it returns to its ground state. Based on the energy level diagrams (which include the vibrational sublevels for each of these two different pigments), we can conclude that generally the excitation rapidly decreases in energy after each intermolecular transfer between dissimilar molecules.

The probability for resonance transfer of electronic excitation decreases as the distance between the two molecules increases. If chlorophyll molecules were uniformly distributed in three dimensions in the lamellar membranes of chloroplasts, they would have a center-to-center spacing of approximately 2.5 nm (25 Å), an intermolecular distance over which resonance transfer of excitation can readily occur (resonance transfer is effective up to about 10 nm for chlorophyll). Thus, both the spectral properties of chlorophyll and its spacing in the lamellar membranes are conducive to an efficient migration of excitation from molecule to molecule by resonance transfer. (See Clayton, Hoch and Knox, or Rabinowitch and Govindjee for a further discussion of resonance transfer.)

Transfers of excitation between photosynthetic pigments. In addition to

the transfer from one Chl *a* molecule to another, excitations can also migrate by resonance transfer from the accessory pigments to Chl *a*. The transfers of excitations among Chl *a*'s can be nearly 100% efficient (i.e., $k_{\text{transfer}} \gg$ rate constants for competing pathways, cf. p. 191), while the fraction of excitation transfers between dissimilar molecules is rather variable. For instance, the transfers of excitations from β -carotene to Chl *a* are very efficient in certain algae, while the fraction of excitation transfers from most xanthophylls to Chl *a* is generally low. The transfer of excitations from α -carotene and lutein to chlorophyll usually is intermediate, e.g., about 40% of the excitations of these carotenoids may be transferred to Chl *a*. Photons absorbed by the carotenoid fucoxanthin—found in brown algae and diatoms—can approach 100% efficiency of excitation transfer to Chl *a*. Also, most excitations of phycobilins and Chl *b* can be transferred to Chl *a*. In red algae, 90% of the electronic excitations produced by the absorption of photons by phycoerythrin can be passed on to phycocyanin and then to Chl *a*; these transfers require about 4×10^{-10} sec each. (See Clayton, Goedheer, or Rabinowitch and Govindjee for efficiencies of excitation transfers between pigments.) We will next consider the direction for excitation transfer between various photosynthetic pigments and then the times involved for intermolecular excitation transfers of chlorophyll.

Some energy is generally lost by each molecule to which the excitation is transferred. Any excess vibrational, rotational, or translational energy is usually dissipated rather rapidly as heat (cf. Fig. 5.8). Therefore, the wavelength positions of the λ_{max} for each type of pigment involved in the sequential steps of excitation transfer tend to become longer in the direction in which the excitation migrates. (The fluorescence emission spectrum of some molecule—which, for transfer to take place efficiently, must appreciably overlap the absorption spectrum of the molecule to which the energy is resonantly transferred—occurs at longer wavelengths than the absorption spectrum of that molecule—cf. Fig. 5.3 for Chl *a*.) Therefore, in order for a second molecule to become excited by resonance transfer, it should have an absorption band at longer wavelengths (lower energy) than the absorption band for the molecule from which it receives the excitation. Thus, the direction for excitation migration by resonance transfer among photosynthetic pigments is usually toward those pigments with longer λ_{max} . We can appreciate this important aspect by considering Figure 5.8. If excitation to the second excited vibrational sublevel of the excited state is the most probable transition predicted by the Franck-Condon principle for each molecule, then the excitation of molecule *A* requires more energy than that of *B* (the pigment to which the excitation is transferred). Hence, λ_{max_A} must

be less than $\lambda_{\max B}$ in the two absorption spectra, which is consistent with our statement that the excitation migrates toward the pigment with the longer $\lambda_{\max}$.

As specific examples of the tendency for excitations to migrate toward pigments with longer $\lambda_{\max}$ in their absorption spectra, we will consider the transfer of excitations from the accessory pigments to Chl *a*. In red algae and in some blue-green algae, phycoerythrin has a $\lambda_{\max}$ at about 560 nm and passes excitation energy on to phycocyanin, which has an absorption maximum near 620 nm. This excitation can then be transferred to the Chl *a* with a $\lambda_{\max}$ near 670 nm (Chl a_{670}). The biliprotein allophycocyanin absorbs maximally at 650 nm and may intervene in the transfer of excitation between phycocyanin ($\lambda_{\max}$ at 620 nm) and Chl a_{670} in some blue-green and red algae. Cryptomonads contain Chl *c* which has a $\lambda_{\max}$ near 650 nm; for such organisms, excitation transfer may be from phycoerythrin ($\lambda_{\max}$ at 560 nm) to phycocyanin (620 nm) to Chl c_{650} and then to Chl a_{670} . Since some of the excitation energy is generally dissipated as heat by each molecule (cf. Fig. 5.8), the excitation represents less energy (longer λ) after each pigment in sequence. Consequently, the overall direction for excitation migration is essentially irreversible, a point to which we will return below (see Clayton, or Rabinowitch and Govindjee).

The light absorption event takes about 10^{-15} sec. This is consistent with the time for an oscillation of the electric field of light, which has a frequency close to 10^{15} cycles/sec (Table 4.1). As the nuclei vibrate back and forth after the absorption of a photon by some electron, their collisions with other nuclei every 10^{-13} sec can lead in such short times to the dissipation of any excess energy in the excited vibrational sublevels. In addition, the radiationless transition from the upper excited singlet to the lower excited singlet of Chl a — $S^b_{(\pi, \pi^*)}$ to $S^a_{(\pi, \pi^*)}$ in Figure 4.5—is completed within 10^{-12} sec. On the other hand, the actual time for the transfer of the excitation between two Chl *a* molecules *in vivo* is somewhat longer—about 1 to 2×10^{-12} sec (see Clayton). Thus, the originally excited chlorophyll molecule usually attains the lowest vibrational sublevel of the lower excited singlet state before the excitation can be transferred to another molecule. The amount of energy resonantly transferred from one Chl *a* molecule to another generally corresponds therefore to the energy indicated by the fluorescence emission spectrum (Fig. 5.3). An excitation representing this amount of energy can in principle be transferred many times by resonance transfer with essentially no further degradation of the energy. The excitation itself is eventually lost from the pool of chlorophyll molecules, assumed here to be identical in spectral properties.

In Chapter 4 we noted that an upper time limit in which processes involving excited singlet states must occur is provided by the kinetics for fluorescence de-excitation. The lifetime for chlorophyll fluorescence from the lower excited singlet state is about 1.5×10^{-8} sec. Thus, time is available for approximately 10,000 transfers of excitation among the Chl *a* molecules—each transfer requiring about 10^{-12} sec—before the loss of the excitation by the emission of fluorescence. The number of excitation transfers between Chl *a* molecules which actually takes place is much less than this, for reasons that will shortly become clear.

Excitation trapping. We have already introduced the special Chl *a*, P_{700} . This pigment absorbs at longer wavelengths than do the other types of Chl *a*, hence the excited singlet state in P_{700} is at a lower energy. It is energetically feasible for Chl a_{680} to excite P_{700} by resonance transfer, but P_{700} does not transfer its excitation to Chl a_{680} to any appreciable extent, i.e., after P_{700} is excited by Chl a_{680} it rapidly loses some of its excitation energy (within 10^{-12} sec), and so does not retain enough energy to re-excite Chl a_{680} by resonance transfer. Therefore, the excited singlet states of other Chl *a* molecules can have their excitations passed on to P_{700} , but not vice versa—analogueous to the irreversibility of the migration of excitations from the accessory pigments to Chl *a*. The excitation resulting from the absorption of radiation by the various photosynthetic pigments is thereby funneled into P_{700} . Such collecting of excitations by one species is the net effect of Equations 5.2 through 5.4 (with the term “trap chl” replaced by P_{700}).

Since one P_{700} is present per 450 chlorophylls, only a few hundred transfers on the average are necessary to get the excitation from Chl *a* to P_{700} . Thus, the 10,000 possible transfers of excitation from one Chl *a* to another do not actually occur. Since each excitation transfer takes approximately 10^{-12} sec, 100 transfers would require about 10^{-10} sec. In agreement with this, both calculations from models and ingenious experimentation have shown that over 90% of the excitations of Chl *a* can migrate to P_{700} (or another trap chl) in less than 10^{-9} sec (see Clayton or Rabinowitch and Govindjee):

The characteristics of fluorescence provide information on the lifetime of the excited singlet state of chlorophyll *in vivo* and thus on the time available for migration of excitations. Specifically, about 3% of the light absorbed by Chl *a* *in vivo* is lost by fluorescence (the actual amount reradiated depends on the illumination level, increasing to about 7% for high illuminations). In other words, the quantum yield for fluorescence *in vivo*, Φ_{Fl} , is approximately 0.03. Equation 4.12 ($\Phi_i = \tau/\tau_i$) indicates that such a quantum yield is equal to τ/τ_{Fl} , where τ is the lifetime of the excited singlet

state and τ_{Fl} is its fluorescent lifetime. Although τ_{Fl} is not known *in vivo*, it is reasonable to assume that it is similar to the fluorescent lifetime of Chl *a* *in vitro*, 1.5×10^{-8} sec. Therefore, Equation 4.12 predicts a lifetime for the excited state of Chl *a* *in vivo* of $(0.03)(1.5 \times 10^{-8} \text{ sec})$, or 0.5×10^{-9} sec. This is another estimate of the average time necessary for the excitation to migrate to the special Chl *a*, P_{700} .

P_{700} acts as a trap for excitations in chloroplast lamellae, while a special type of bacteriochlorophyll with a λ_{max} between 870 and 890 nm acts in an analogous manner in bacteria. One of the useful features of such excitation traps is to have an excited singlet state lower in energy than the excited singlet states in the other pigment molecules. This lower energy state (longer λ_{max} for absorption) is a consequence of the molecular environment in which the chlorophyll molecule acting as an excitation trap is located. Moreover, the longer λ_{max} ensures the directionality for the migration of excitations. Another characteristic of special molecules like P_{700} is their relative rarity. Thus, most of the photosynthetic pigments act as antennae, or light harvesters, which collect the radiation and channel the excitations toward the trap chl, as illustrated in Figure 5.9. Processing of the excitation originally caused by light takes place only at the trap chl's. This participation in the essentially irreversible, electron-transfer process is the crucial feature of an excitation trap.

When the excitation migrates to the trap chl P_{700} , this special Chl *a* goes to an excited singlet state, as would any other Chl *a*. Since P_{700} cannot readily excite other chlorophylls by resonance transfer, it might become de-excited by the emission of fluorescence. However, very little fluorescence of the trap chl's is observed *in vivo*, which could be explained by a transition to an excited triplet state or de-excitation by a photochemical event (cf. Eq. 5.5, $\text{trap chl}^* + A \rightarrow \text{trap chl}^+ + A^-$). Although the relatively long lifetime of a triplet would allow time for a photochemical reaction to take place, such as the reduction of *A* in Figure 5.9, there is no direct evidence for a triplet state of P_{700} *in vivo*. On the other hand, the rapid donation (within 10^{-10} sec) of an electron to an acceptor could prevent the fluorescent de-excitation of P_{700} , and this is apparently the case *in vivo*.

As we have just indicated, an excited trap chl can rapidly donate an electron to some acceptor molecule, which is part of the *photochemistry* of photosynthesis. The donation of the electron initiates the *chemical* reactions of photosynthesis and the subsequent storage of energy in stable chemical bonds. Moreover, once the trap chl has lost an electron, it can take on another from some donor, indicated by *D* in Equation 5.6 ($\text{trap chl}^+ + D \rightarrow \text{trap chl} + D^+$) and Figure 5.9. Thus, the photochemical

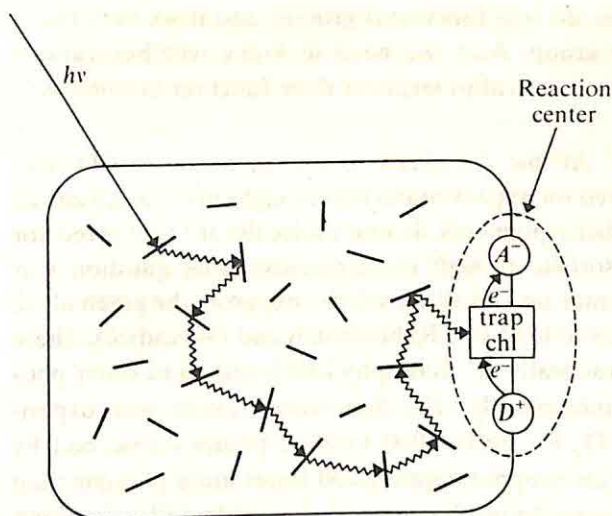


Figure 5.9

Schematic indication of a group of pigments functioning together as a photosynthetic unit which harvests a light quantum ($h\nu$) and passes the excitation on to a special trap chlorophyll. In the reaction center an electron (e^-) is transferred from the trap chl to some acceptor (A^- in the reduced form) and then replaced by another electron coming from a suitable donor (D^+ in the oxidized form).

reactions of photosynthesis lead to electron flow. An excitation trap such as P_{700} plays a key role in the conversion of radiant energy into forms of energy which are biologically useful. We generally refer to the trap chl plus A and D as a *reaction center*, illustrated in Figure 5.9 (see Pearlstein).

Photosynthetic units and enhancement effects

So far, we have discussed the absorption of light by photosynthetic pigments and the ensuing transfers of excitation among these molecules, and this leads us to a consideration of whether there are discrete units of such pigments acting together in some concerted fashion. Such an ensemble was presented in Figure 5.9, where we indicated that the light-harvesting photosynthetic pigments greatly outnumber the special trap chl molecules, the latter possibly occurring in a one-to-one relationship with suitable electron acceptors and donors. The questions we will consider in this section are whether photosyn-

thetic pigments are organized into functional groups, and if so, how many molecules make up one group. Also, we need to know whether various groupings of pigments are identical in terms of their function in photosynthesis.

Photosynthetic units. At low illuminations one molecule of CO_2 can be fixed and one O_2 evolved for approximately every eight photons absorbed by any of the photosynthetic pigments. Is one molecule of O_2 evolved for every eight photons absorbed at *high* illuminations? This question was answered in 1932 by R. Emerson and W. Arnold by exposing the green algae *Chlorella* to intense flashes of light (see Rabinowitch and Govindjee). These intense flashes excited practically all chlorophyll molecules and other photosynthetic pigments simultaneously. The maximum yield in such experiments was one evolved O_2 for every 2000 to 2500 photons absorbed by chlorophyll molecules! This is approximately 250 times more photons than the eight which are necessary to produce one oxygen at low illuminations. At low levels of illumination there is sufficient time between the arrival of individual photons so that the excitations of the accessory pigments and Chl *a* can be efficiently collected in a trap chl and used for the chemical reactions of photosynthesis. At high illuminations, on the other hand, many photosynthetic pigments become excited at the same time, and only one excited chlorophyll in as many as 250 molecules leads to any photochemical reaction. It is as if there were a unit of an average of 250 chlorophyll molecules acting together. When any one molecule in this *photosynthetic unit* becomes excited by light and then has its excitation transferred to the reaction center, the concomitant excitation of other chlorophyll molecules in that unit cannot be used for photosynthesis.

The above conclusion on photosynthetic units can also be considered in terms of Figure 5.9, where the trap chl is shown interacting with the electron acceptor *A* and donor *D*. The rate-limiting step for photosynthesis is not light absorption, excitation transfer, or photochemistry (electron donation by trap chl*), but the subsequent steps leading to O_2 evolution and CO_2 fixation. A brief intense illumination thus leads to more excitations than can be processed by the electron transfer reactions associated with a photosynthetic unit. In the limit of a very intense flash simultaneously exciting all photosynthetic pigments in some unit, one excitation is processed and all others are dissipated by various nonphotochemical de-excitation processes, such as those discussed in the previous chapter.

A photosynthetic unit of approximately 250 chlorophylls may well be both a functional and a structural entity in chloroplasts. Chloroplast lamellae have subunits calculated to contain 230 chlorophyll molecules. These might

actually be photosynthetic units, although their electron transfer reactions—e.g., Equations 5.5 and 5.6—remain to be demonstrated. In some photosynthetic bacteria, 40 bacteriochlorophylls form a photosynthetic unit having 1 trap chl. The size of the bacterial photosynthetic unit is rather variable and often larger than this. Indeed, the number of chlorophylls acting together as a photosynthetic unit in the chloroplasts of higher plants also varies (see Clayton, as well as the article by Schmid and Gaffron in Pearlstein).

Excitation processing by photosynthetic units. The electron excitation originally caused by the absorption of a photon can be processed by the chemical reactions associated with a photosynthetic unit about once every 0.01 sec (see Clayton). This processing time has important consequences for both the efficiency of light use at different illumination levels and the optimal size of a photosynthetic unit.

The highest illumination normally encountered by plants is when the sun is directly overhead on a cloudless day (approximately 100,000 lux), in which case the photon flux in the visible region is about 1.2×10^{17} photons/cm²-sec (p. 172). The average chlorophyll concentration in chloroplasts is near 25 mM (p. 201), and in passing through a thickness of 2 μ m (2×10^{-4} cm) about $\frac{1}{4}$ of the incident photons in the visible region are absorbed by a single chloroplast. Using these numbers, we can estimate how often an individual chlorophyll molecule might absorb a photon when a leaf is exposed to direct sunlight. Specifically, $(\frac{1}{4})(1.2 \times 10^{17})$, or 3.0×10^{16} photons/cm²-sec, would be absorbed by a single chloroplast containing about $(2 \times 10^{-4} \text{ cm})(25 \times 10^{-6} \text{ moles of chlorophyll/cm}^3)(6.023 \times 10^{23} \text{ molecules/mole})$, or 3.0×10^{15} molecules of chlorophyll/cm². Consequently, the rate of absorption is about $(3.0 \times 10^{16} \text{ photons absorbed/cm}^2\text{-sec})/(3.0 \times 10^{15} \text{ molecules of chlorophyll/cm}^2)$, or 10 photons absorbed per sec by each chlorophyll molecule in a chloroplast exposed to 100,000 lux.

As we have just calculated, each chlorophyll in an unshaded chloroplast could absorb a photon on the average about once every 0.1 sec. If there are 250 chlorophylls per photosynthetic unit, 25 of these molecules would be excited every 0.01 sec. However, since the processing time per photosynthetic unit is 0.01 sec, only one of these 25 excitations could be used photochemically—the other 24 are dissipated by nonphotochemical de-excitation reactions. Thus, although the chemical reactions will operate at their maximum rate under such conditions of high illumination, a large fraction of the electronic excitations caused by light absorption will not be used for photosynthesis.

Full midday sunlight is seldom incident on a chloroplast under natural conditions, since chloroplasts are generally shaded by other chloroplasts in

the same cell, by plastids in other cells, and perhaps by overlying leaves. Furthermore, the amount of sunlight incident on a plant is much less at sunrise or sunset and on overcast days than at midday. For the sake of argument, let us consider a situation when the light incident on a chloroplast is twenty-five times less than it is from the direct midday sun, namely an illumination of approximately 4000 lux. In this case, each chlorophyll molecule in the chloroplast absorbs a photon once every 2.5 sec, which is a much longer time between the arrival of photons than the time required by a photosynthetic unit for processing an electronic excitation. However, when individual chlorophylls are excited every 2.5 sec at this moderate illumination of 4000 lux, some chlorophyll molecule in a photosynthetic unit of 250 chlorophylls will become excited on the average of once every 0.01 sec (2.5 sec/250 excitations in a unit means 0.01 sec/single excitation in a unit). This excitation frequency per photosynthetic unit is such that the light can be efficiently used for photosynthesis. In other words, the photons are arriving at a rate such that the excitations produced by essentially all of them can be used, and, moreover, the chemical reactions are working at their maximum capacity. Consequently, a typical photosynthetic unit with its associated photochemistry and enzymatic reactions will function very effectively at a moderate illumination level.

What would happen if there were but one chlorophyll molecule per photosynthetic unit? If the chemical reactions required 0.01 sec as above, this single pigment molecule would be excited about once every 2.5 sec at an illumination of 4000 lux and the excitation could easily be processed by the chemical reactions. However, the photochemical step plus the subsequent enzymatic reactions would be working at only 0.4% of capacity—(0.01 sec)/(2.5 sec), or 0.004, is the fraction of time they would be used. In other words, although all the absorbed photons would be used for photosynthesis, even the slowest of the chemical steps would be idle over 99% of the time. Given the 0.01 sec processing time for the chemical reactions, the grouping of 250 chlorophylls into one photosynthetic unit connected with the appropriate enzyme machinery provides a plant with an efficient mechanism for handling the usual illuminations—both in harvesting the photons and using the chemical reactions at a substantial fraction of their capacity.

Photosynthetic action spectra and enhancement effects. The electronic excitations resulting from light absorption by any of the pigments in a photosynthetic unit can be transferred to the trap chl and thus lead to photochemical reactions. Therefore, it would be reasonable to expect that the absorption spectrum of chloroplasts would fairly well match the action spectrum for photosynthesis. However, the action spectrum for CO_2 fixation

or O_2 evolution and the overall absorption spectrum for the photosynthetic pigments in the same organism generally do not coincide. Among other things, there is a so-called “red drop”—the photosynthetic action spectrum drops off much more rapidly in the red region beyond 690 nm than does the absorption spectrum for chlorophylls and other pigments—as illustrated in Figure 5.10 for the case of a green alga.

R. Emerson showed in 1957 that the relatively low photosynthetic efficiency of *Chlorella* in far-red light—a “red drop” such as that in Figure 5.10—could be increased by simultaneously using light of a shorter wavelength in addition to the far-red light (see Emerson). The photosynthetic rate with the two beams could be 30% to 40% greater than the sum of the rates of far-red light and the shorter wavelength light used separately! We can also describe the enhancement in terms of quantum yield (see p. 191 for a definition of quantum yield). For example, we could determine the quantum yield for O_2 evolution by *Chlorella* using 700 nm radiation in both the presence and absence of 650 nm light. In such an experiment the quantum yield using the 700 nm radiation is higher when the 650 nm light is also present (see Myers). Such synergism, or enhancement, by two wavelengths of light suggests that photosynthesis involves the cooperation of two distinct photochemical reactions. The light of wavelengths greater than 690 nm might mainly power only one of the two necessary reactions, and thus photosynthesis would not proceed at an appreciable rate. When shorter wavelengths are also used, the other necessary reaction could take place, resulting in a marked enhancement of the photosynthetic rate or quantum yield (see Gregory).

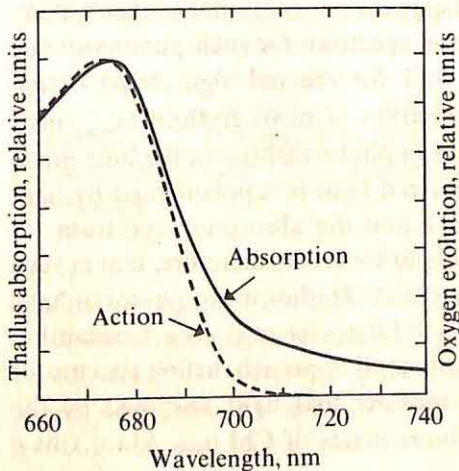


Figure 5.10

Absorption spectrum for an algal thallus and the action spectrum for its oxygen evolution, illustrating the “red drop” in photosynthesis. (Data are for *Ulva taeniata* and are taken from F. T. Haxo and L. R. Blinks, *Journal of General Physiology* 33: 389–422 (1950). Used by permission.)

Other observations further indicate two distinct photochemical systems. For example, since photosynthesis is enhanced by adding a shorter wavelength to far-red illumination, a smaller percentage of light should then be reradiated as fluorescence. Indeed, the two wavelengths together evoke less fluorescence than the two acting separately. Also, a brief transient change in the rate of O_2 evolution is observed when *Chlorella* is switched from 650 nm to 700 nm light (for convenience both beams can be adjusted to give the same rate of O_2 evolution). A transient in the opposite direction is observed when the cells are returned to the 650 nm illumination. Such effects are consistent with having two photochemical systems in the photosynthetic membranes, both of which are necessary for O_2 evolution. Further evidence is provided by the actual isolation of two distinct photochemical systems (see Boardman). An important question remains, however: Under natural conditions, are excitations transferred between the two systems to any significant extent?

Composition of the two photosystems. We can use the photosynthetic enhancement effect to study the pigments in each of the two photochemical systems involved in photosynthesis. The system containing the pigments absorbing beyond 690 nm is referred to as Photosystem I, a terminology introduced by L. M. N. Duysens, J. Ames, and B. M. Kamp in 1961 (see Clayton; Duysens *et al.*; or Kamen). Much of the far-red absorption by Photosystem I is due to a type of Chl *a* with a $\lambda_{\max}$ near 680 nm, Chl a_{680} . The special Chl *a*, P_{700} , is found exclusively in Photosystem I. Therefore, light above 690 nm is absorbed mainly by Chl a_{680} and P_{700} in Photosystem I.

An action spectrum for the enhancement of photosynthesis in the presence of a constant irradiation with wavelengths longer than 690 nm, which are absorbed by Photosystem I, should indicate the pigments in the other system, Photosystem II. For example, the action spectrum for such photosynthetic enhancement represented in Figure 5.11 for the red alga *Porphyridium cruentum* resembles the absorption spectrum of phycoerythrin ($\lambda_{\max}$ near 540 nm, cf. Fig. 5.7). A marked increase in photosynthesis in the blue-green alga *Anacystis nidulans* occurs when far-red light is supplemented by light absorbed by phycocyanin (cf. Fig. 5.11 and the absorption spectrum for phycocyanin in Fig. 5.7). It is reasonable to conclude, therefore, that in such algae the phycobilins are in Photosystem II. Studies using photosynthetic enhancement also indicate that Chl *b* is in Photosystem II, as is fucoxanthin in the brown algae. In a related experimental approach, action spectra for the evocation of Chl *a* fluorescence indicate that light absorbed by the phycobilins and Chl *b* leads to the fluorescence of Chl a_{670} . Since Chl *b*

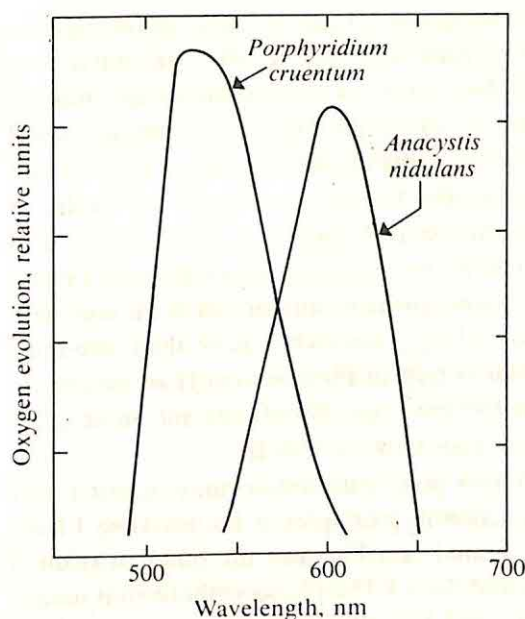


Figure 5.11

Action spectra for the enhancement of O_2 evolution for two algae. Cells were exposed to a constant red illumination (wavelengths beyond 690 nm) plus a specific photon flux at the various wavelengths indicated on the abscissa. The ordinate represents the rate of O_2 evolution for the two beams acting together minus that produced by the shorter wavelength acting alone. The pigment absorbing the light leading to enhancement of O_2 evolution in *Porphyridium cruentum* is phycoerythrin, while phycocyanin is responsible in *Anacystis nidulans*—cf. absorption spectra in Figure 5.7. (Source: R. Emerson and E. Rabinowitch, *Plant Physiology* 35: 477–485 (1960). Data used by permission.)

and the phycobilins are in Photosystem II, Chl a_{670} is most likely also in Photosystem II. Although carotenoids occur and function predominantly in Photosystem II, individual types may be associated with either or both photosystems (see Clayton; Fork and Ames; Goedheer; Myers; or Rabinowitch and Govindjee).

Photosystems I and II in the chloroplast grana of higher plants appear to be structurally distinct entities, each with 200 to 300 light-harvesting chlorophylls and a special chlorophyll acting as an excitation trap. In other words, each photosystem acts like one of the photosynthetic units discussed above. Moreover, each of the photosystems can be separately isolated and its components chemically determined (see Boardman, as well as Rabinowitch and Govindjee). Photosystem I contains a few hundred Chl a_{680} molecules, very little or no Chl b , one P_{700} , and probably some carotenoids as accessory pigments. The photosynthetic unit known as Photosystem II has most of its chlorophyll as Chl a_{670} , somewhat more than one-third as Chl b , and no P_{700} —the excitation trap in Photosystem II is apparently a special Chl a with a $\lambda_{\max}$ near 690 nm. The phycobilins and most other accessory pigments are associated with Photosystem II.

Although Photosystem II of higher plant chloroplasts may function only in the grana, two distinct and functioning examples of Photosystem I have recently been identified, one in granal lamellae and the other in stromal lamellae (cf. Fig. 1.9). (See Park and Sane.) The photosynthetic unit identified as Photosystem I in the stromal lamellae appears to contain fewer chlorophyll molecules than the similar unit in the granal lamellae. Blue-green algae also have Photosystems I and II, but they do not contain any Chl b . The number of photosystems in photosynthetic bacteria is not universally agreed upon (see Frenkel 1970). Bacteria are unique among photosynthetic organisms in oxidizing primarily organic acids or inorganic sulfur-containing compounds instead of water; hence, oxygen evolution does not accompany photosynthesis by bacteria.

The trap chl in any of the photosynthetic units must be accessible to the light-harvesting pigments and to the electron transfer components which become oxidized or reduced. This trap chl, together with the associated species which accept electrons from it or donate electrons to it, is generally referred to as a reaction center (cf. Fig. 5.9). The reaction center is the locus for the photochemistry of photosynthesis (Eq. 5.5) and is located at or near the surface of the photosynthetic unit, where it can interact with the compounds involved with subsequent electron transfer.

Electron flow and photophosphorylation

The photochemical reaction of photosynthesis involves the removal of an electron from an excited state of the special chlorophyll that acts as an

excitation trap. The movement of the electron from this trap chl to some acceptor begins a series of electron transfer steps which can ultimately lead to the reduction of NADP. The oxidized trap chl, which has lost an electron, can accept another from some donor, as in the steps which lead to oxygen evolution. Coupled to the electron transfer reactions in chloroplasts is the formation of ATP, although the actual mechanism for this "photophosphorylation" is as yet unknown. In this section we will consider some of the components of chloroplasts involved in accepting and donating electrons—a discussion of the energetics of such processes will follow in the next chapter.

The various steps of photosynthesis occur over a wide range of times (see Frenkel 1970 and Kamen). For instance, the absorption of light and the transfer of excitations from both accessory pigments and Chl *a* molecules to the trap chl occupy a time interval from 10^{-15} to 10^{-9} sec after the arrival of the photons. The photochemical event at the reaction center, which can be described by Equation 5.5, may take 10^{-10} sec. Such separation of an electron from the trap chl leads to a bleaching—a decrease in the absorption coefficient for wavelengths in the visible region—of this pigment. By observing the kinetics of this bleaching in the far-red region, we can determine the occurrence of photochemistry, which signals that the excitation caused by absorption of radiant energy has led to an increase in chemical energy. The donation of an electron to the oxidized trap chl (Eq. 5.6) usually occurs 10^{-6} to 10^{-5} sec after the arrival of the photons, and restores the original spectral properties of the trap chl. The ensuing electron flow to some components can last into the millisecond range. Finally, the overall processing time for a photosynthetic unit plus the associated enzymes is about 0.01 sec per excitation.

Electron flow. Studies on electron flow accompanying photosynthesis were greatly advanced in 1938 when R. Hill demonstrated that isolated chloroplasts could evolve oxygen and reduce certain exogenous compounds in the light. Oxygen evolution proceeded in the absence of CO_2 , suggesting that CO_2 fixation and O_2 evolution were (contrary to the prevailing belief) separate processes. Using ^{18}O -labeled H_2O and ^{18}O -labeled CO_2 in separate experiments, S. Ruben and M. D. Kamen conclusively showed in 1941 that the evolved oxygen comes from water and not from CO_2 . Subsequent studies have elucidated many of the steps intervening between O_2 evolution and CO_2 fixation in photosynthesis.

Our point of departure for the present discussion of electron flow in photosynthesis will be to indicate the overall accomplishments of the process.

Although the actual mechanism of this water oxidation is still unclear, it can be represented as follows:



where H in Equation 5.8 represents $\text{H}^+ + \text{e}^-$. (Four molecules of water may be involved in the evolution of one molecule of oxygen, in which case the reaction might more realistically be written as $4\text{H}_2\text{O} \longrightarrow \text{O}_2 + 4\text{H} + 2\text{H}_2\text{O}$.) The process of CO_2 fixation into a carbohydrate involves the reduction of carbon. Four hydrogen atoms are required per carbon atom:



where $\{\text{CH}_2\text{O}\}$ represents a general carbohydrate, as indicated previously (Fig. 5.1). The movement of the reductant H in Equations 5.8 and 5.9 can be conveniently followed by tracing the flow of the electrons ($\text{H} = \text{H}^+ + \text{e}^-$), which explains why our topic here is electron flow and not hydrogen movement. Moreover, reducing a compound is chemically equivalent to adding electrons, while oxidation is the removal of electrons.

Some pathway for photosynthetic electron flow is needed in order to have the H's indicated as products in Equation 5.8 become the H's required as reactants in Equation 5.9. This electron transfer pathway involves both Photosystems I and II as well as a number of electron-accepting and donating components. The oxidant involved with oxygen evolution (Eq. 5.8) is provided by Photosystem II. The primary oxidant is trap chl⁺, which leads to the oxidation of water (cf. Eq. 5.6, $\text{trap chl}^+ + \text{D} \longrightarrow \text{trap chl} + \text{D}^+$). The reductant required for carbon reduction (Eq. 5.9) is produced by the excited trap chl in Photosystem I (cf. Eq. 5.5, $\text{trap chl}^* + \text{A} \longrightarrow \text{trap chl}^+ + \text{A}^-$). These two photosystems are linked by a chain of components along which there is a transfer of electrons, as illustrated in Figure 5.12.

For each photon absorbed by any of the accessory pigments or Chl *a*'s in a photosynthetic unit, one electron can be removed from its trap chl. Since 4 electrons are involved per O_2 derived from water, the evolution of this 1 molecule of oxygen requires the absorption of 4 photons by Photosystem II (cf. Eq. 5.8 and the 4 photons incident on Photosystem II in Fig. 5.12). An additional 4 photons absorbed by Photosystem I are required for the reduction of the 2 molecules of NADP which are necessary for the subsequent reduction of 1 CO_2 molecule (Eq. 5.9 and Fig. 5.12). Hence, 8 photons are needed for the evolution of 1 molecule of O_2 and the fixation of 1 molecule of CO_2 . (In the next chapter we will consider how many photons are used to provide the actual number of ATP's required per CO_2 fixed.) Although there are alternatives to the series representation proposed

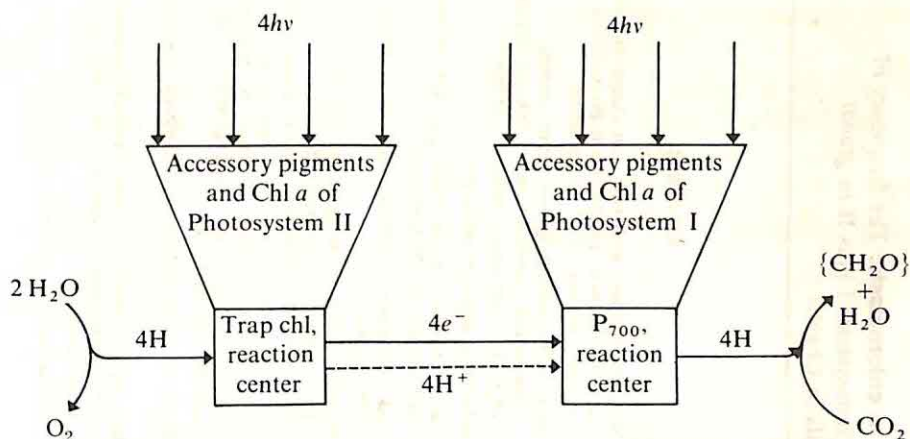


Figure 5.12

Schematic model for a series representation of the two photosystems of photosynthesis, indicating the stoichiometry of various factors involved in the reduction of a CO_2 molecule to a carbohydrate ($\{\text{CH}_2\text{O}\}$).

by R. Hill and F. Bendall in 1960 (Fig. 5.12), the scheme takes into consideration the results of many investigators and has generally become accepted as an overall description of electron flow in chloroplast grana.

Components of the electron transfer pathway. We shall now turn our attention to the specific molecules which act as electron acceptors or donors in chloroplasts. A summary of the characteristics of the better-known components of the pathway is presented in Table 5.1. We will begin our discussion by considering the photochemistry at the reaction center of Photosystem II, and then proceed with a consideration of the various substances in the approximate sequence in which they are involved in electron transfer along the pathway from Photosystem II to Photosystem I. We will conclude by considering the fate of the excited electron in Photosystem I.

The electron removed from the excited trap chl in the reaction center of Photosystem II is replaced by one coming from water in the process which leads to oxygen evolution. The molecular details of this water oxidation are unknown at present (see Bishop, Cheniae, Heath, or Pearlstein). Manganese may be involved, presumably while bound to some protein. Chloride is also implicated in the oxygen evolution steps. The electron from trap chl* of Photosystem II is transferred by a series of molecules comprising the photosynthetic "electron transfer chain," a term describing the pathway from Photosystem II to Photosystem I (see Fig. 5.12). All of the components involved with electron transfer in this chain of molecules are probably not

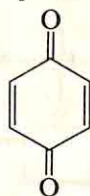
Table 5.1
Representative properties of some of the components involved with electron transfer in chloroplasts. The frequency of components is calculated per 450 chlorophylls, the approximate number of chlorophylls in Photosystems I plus II in grana lamellae (see Bishop, Boardman, as well as Hind and Olson). We will discuss redox potentials in Chapter 6.

Name	Molecular weight	Approximate number/450 chlorophylls	Numbers of electrons accepted or donated per molecule	Approximate midpoint redox potential in mv	Comment
Ferredoxin	12,000	5	1	-430	Nonheme protein with two iron atoms/molecule; accepts electrons excited from Photosystem I, perhaps by way of an intermediate.
Ferredoxin-NADP reductase	42,000	?	...	...	An enzyme containing one flavin adenine dinucleotide/molecule (see p. 000 for structure of FAD); apparently bound to lamellae.
NADP-NADPH ₂	743-745	30	2	-320	Soluble in aqueous solutions.
Cyt <i>b</i> ₅₆₄	?	2	1	-30	May be involved in the cyclic pathway between ferredoxin and Photosystem I.
Cyt <i>b</i> ₅₅₉	?	2	1	(55)	Peak absorption at 559 nm; may be bound to Photosystem II.
Plastoquinone A	748	30	2	113	Probably acting at the reducing end of Photosystem II; can be oxidized by Photosystem I; there are a total of about 90 quinones/450 chlorophylls; only some of the Plastoquinone A molecules may be involved in electron transport and Plastoquinone C may also be involved.
Cyt <i>f</i>	13,000 to 60,000 per heme	1	1	360	In or bound to Photosystem I, which can oxidize it.
Plastocyanin	21,000	1	2	370	Blue protein (reduced form is colorless) associated with Photosystem I; contains two copper atoms/molecule.
P ₇₀₀	893	1	1	430	A Chl <i>a</i> acting as the trap chl of Photosystem I.

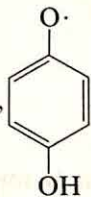
known, and some of the molecule-to-molecule sequences for electron transfer are uncertain.

One of the first molecules to accept the electrons donated by the excited trap chl of Photosystem II is a plastoquinone. Chloroplast lamellae actually

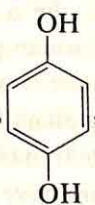
contain eight or more different types of quinones.



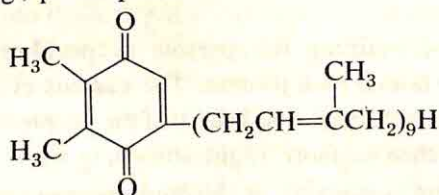
is the structure

of quinone. This becomes a semiquinone, , when one hydrogen atom

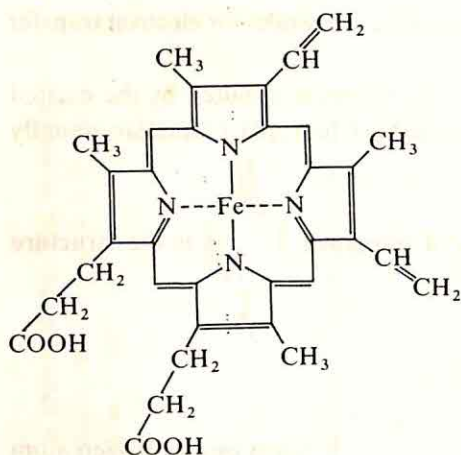
is added (a semiquinone is a free radical because of the presence of an

unpaired electron), and a hydroquinone, , when two are added. There

is great variety among quinones because of substituents attached to the ring, e.g., plastoquinone A is



Like chlorophyll, plastoquinone A has a nonpolar terpenoid or isoprenoid tail, which may serve to anchor it at the proper location in the lamellar membranes of chloroplasts. When donating or accepting electrons, plastoquinones have characteristic absorption changes in the UV near 250 to 260 nm which can be monitored to study their electron transfer reactions (see Bishop or San Pietro for further details on quinones). From the plastoquinone, electrons may be passed on to the *b* cytochrome having a $\lambda_{\max}$ at 559 nm. Electrons then move energetically downhill to a blue protein known as plastocyanin or to cytochrome *f*. (The energetics of electron transfer is discussed in Ch. 6, cf. Fig. 6.4.)

**Figure 5.13**

The iron-containing porphyrin known as *heme*, the chromophore for cytochrome *c*.

Cytochromes are extremely important components of electron transfer pathways in chloroplasts and mitochondria. They have three absorption bands in the visible region; the α , β , and γ bands. (As far as we know, absorption of light by cytochromes plays no part in photosynthesis.) In 1925 D. Keilin described three different types of cytochrome based on the spectral position of their α (long) wavelength band. Cytochromes of the *a* type have a $\lambda_{\max}$ for the α band from 600 to 605 nm, *b* types near 560 nm, and *c* types near 550 nm. All cytochromes have β bands near 515 to 530 nm. The main short wavelength band, the γ or Soret band, usually has a $\lambda_{\max}$ between 415 and 430 nm (see Bishop; Davies *et al.*; or San Pietro for further information on plant cytochromes).

Cytochromes consist of an iron-containing tetrapyrrole or porphyrin known as *heme* (Fig. 5.13), which is bound to a protein. The various cytochromes differ in both the substituents around the periphery of the porphyrin ring and the protein to which the chromophore (light-absorbing part) is attached. Cyt *f** is a *c*-type cytochrome occurring in chloroplasts, and contains the chromophore indicated in Figure 5.13. Many different hemo-proteins of the Cyt *b* type are found in plants, two occurring in chloroplasts (cf. Table 5.1). These various *b* cytochromes appear to have the same chromophore attached to different proteins, and thus their individual absorption properties are probably due to changes in the protein. Like the cytochromes, chlorophylls are tetrapyrroles (cf. the structure of Chl *a* in Fig. 5.2), but they have a Mg atom in the center of the porphyrin ring while

**f* from *frons*, the Latin for leaf.

cytochromes have an Fe. Furthermore, the acceptance or donation of an electron by a cytochrome involves a transition between the two states of its iron, Fe^{++} and Fe^{+++} , whereas the electron removed from chlorophyll in the photochemical reactions of photosynthesis is most likely one of the π electrons in the conjugated system of the porphyrin ring.

Instead of the usual valence bonds, the metal atoms in chlorophylls and cytochromes should really be presented in terms of the six coordinate bonds described by ligand-field (molecular-orbital) theory. Fe has one coordinate bond to each of the four N's in the porphyrin ring (the two solid and two broken lines emanating from Fe in Fig. 5.13), one to an N in the imidazole side chain of a histidine, and another to the S in a methionine, both of which occur in the protein to which the chromophore is bound (one bond is above and one is below the plane of the porphyrin in Fig. 5.13). (See Dickerson.) The donation of an electron by the ferrous form of Cyt *c*, ferrocycytochrome *c* (Fig. 5.13), causes the iron to go to the ferric state, Fe^{+++} . The extra positive charge on the iron in ferricytochrome *c* can either attract anions such as OH^- or be delocalized to adjacent parts of the molecule. The six coordinate bonds remain in ferricytochrome *c* and the conjugation in the porphyrin ring is only moderately changed from that in ferrocycytochrome *c*. Hence, the extensive bleaching of chlorophyll which follows the loss of an electron from its porphyrin ring does not occur with electron donation by the iron atom in cytochrome. Instead, the transition from Fe^{++} to Fe^{+++} leads to less of a change in the absorption bands, since electrons in the ring conjugation are influenced only secondarily. Nevertheless, such changes in absorption upon the acceptance or donation of electrons by cytochromes are important (see Davies *et al.*).

Spectral properties of cytochromes can be used for monitoring electron movement *in vivo* as well as for cytochrome identification. The removal of an electron from a cytochrome (oxidation) causes its three absorption bands to become less intense, broader, and to shift toward shorter wavelengths. The absorption coefficients at λ_{max} in the reduced form are about 3×10^4 liters/mole-cm for the α band, somewhat less for the β band, and over 10^5 liters/mole-cm for the γ (Soret) band. Upon oxidation, the ϵ_λ for the α band decreases just over 50%, while smaller fractional changes generally occur in the absorption coefficients of the β and γ bands (the λ_{max} is also at shorter wavelengths in the oxidized form). Such absorption changes permit a study of the kinetics of electron transfer while the cytochrome molecules remain embedded in the internal membranes of chloroplasts or mitochondria. By monitoring the spectral changes of the chloroplast cytochromes we can show that the electron movement along the electron transfer chain between

Photosystem II and Photosystem I (Figs. 5.12 and 6.4) occurs 10^{-5} to 10^{-3} sec after light absorption by some pigment molecule in Photosystem II.

Electrons which have traversed the electron transfer chain from Photosystem II to plastocyanin or Cyt *f* can be accepted by the trap chl of Photosystem I, P_{700} , if the latter is in the oxidized form (cf. Eq. 5.6: $\text{trap chl}^+ + D \rightarrow \text{trap chl} + D^+$). The photochemical change in P_{700} can be followed spectrophotometrically since the loss of an electron causes a bleaching of both its Soret and red absorption bands; the subsequent acceptance of an electron restores the original spectral properties. P_{700} is bleached (oxidized) by light absorbed by Photosystem I and then restored (reduced) following the absorption of photons by Photosystem II. Thus, the electron removed from P_{700} is replaced by one coming from Photosystem II by means of the electron transfer chain (Figs. 5.12 and 6.4). Also, far-red light (above 690 nm) absorbed by Photosystem I leads to an oxidation of Cyt *f*, indicating that an electron from Cyt *f* can be donated to P_{700} .

The electron from P_{700}^* reduces the iron-containing protein, ferredoxin, perhaps by way of an intermediate compound (see Ke). Ferredoxin contains two irons in the ferric state bound by sulfide bonds; the electron from the lower excited singlet state of P_{700} causes the reduction of one of these ferric atoms to the ferrous form. By means of the enzyme ferredoxin-NADP reductase, two molecules of ferredoxin reduce one molecule of NADP to yield NADPH_2 . (We will discuss NADP and NADPH_2 in the next chapter.)

Types of electron flow. We can distinguish among three different types of photosynthetic electron transfer, or flow, each one depending on the compound to which electrons are transferred from ferredoxin (see Arnon). In *noncyclic* electron flow electrons coming originally from water are ultimately used to reduce NADP. The overall noncyclic electron flow is as follows: an electron from water goes to the trap chl^+ of Photosystem II; there it replaces a donated electron which moves along the electron transfer chain to the oxidized P_{700} in Photosystem I; the electron from P_{700} moves to ferredoxin and then to NADP. Such noncyclic electron flow follows essentially the same pathway as the reductant H moving from left to right in Figure 5.12 (see also Fig. 6.4).

Electrons from ferredoxin may also reduce oxygen, which yields H_2O_2 and eventually H_2O ($\text{O}_2 + 2e^- + 2\text{H}^+ \rightleftharpoons \text{H}_2\text{O}_2 \rightleftharpoons \text{H}_2\text{O} + \frac{1}{2}\text{O}_2$). Since equal amounts of oxygen are evolved at Photosystem II and then consumed using reduced ferredoxin in a quite separate reaction, such electron flow is termed *pseudocyclic* (illustrated in Fig. 6.4). There is no net oxygen change accompanying pseudocyclic electron flow, although it is not a cycle in the sense of having electrons cyclically traverse a certain pathway. It is not yet

clear to what extent pseudocyclic electron flow occurs *in vivo*, if at all, although such electron flow can readily be demonstrated with isolated chloroplasts.

In *cyclic* electron flow the electron from the reduced form of ferredoxin moves back to the electron transfer chain between Photosystems I and II and eventually reduces an oxidized P_{700} . Cyt b_{564} may act as an intermediate for this cyclic electron flow by intervening between ferredoxin and the electron transfer chain (cf. Fig. 6.4). Cyclic electron flow does not involve Photosystem II at all, hence it can be caused by far-red light absorbed only by Photosystem I—a fact which is often exploited in experimental studies. When far-red light absorbed by Photosystem I is used, cyclic electron flow can occur but noncyclic does not, so that no $NADPH_2$ is formed and no O_2 is evolved (cyclic electron flow can lead to the formation of ATP, as we will show in the next chapter). When light absorbed by Photosystem II is added to cells exposed to far-red illumination, both CO_2 fixation and O_2 evolution can proceed, and photosynthetic enhancement is achieved. Treatment of chloroplasts or plant cells with the oxygen evolution inhibitor DCMU—3-(3,4-dichlorophenyl)-1,1-dimethyl urea—also leads only to cyclic electron flow; DCMU therefore has many applications in the laboratory and is also a convenient herbicide. Since only Photosystem I activity occurs in the stromal lamellae, presumably only cyclic electron flow occurs there.

Many questions concerning electron transfer in photosynthesis remain unanswered. For instance, what are the intermediates involved in the evolution of oxygen from water? Does the removal of an electron from each of the two or four water molecules involved in the evolution of one O_2 molecule normally occur at the reaction center of a single Photosystem II, or are two or perhaps four different photosynthetic units involved? As indicated in Table 5.1, ferredoxin, the cytochromes, and P_{700} can accept or donate only one electron. These molecules must interact with NADP, plastocyanin, and the plastoquinones, all of which can transfer two electrons at a time. Do the two electrons which reduce plastoquinone and plastocyanin sequentially come from the same Photosystem II, or do they come from two different ones? Similarly, do the two electrons used to reduce NADP traverse the same or different electron transfer chains? Both the pyridine nucleotides and the plastoquinones are considerably more numerous than other molecules involved with photosynthetic electron flow (Table 5.1), and this probably has important implications for the electron transfer reactions. Moreover, NADP is soluble in aqueous solutions and so could diffuse to the NADP-ferredoxin reductase where two electrons are transferred to it to yield $NADPH_2$. Although a great deal remains to be learned about photosynthetic

electron flow, much more physical and chemical information describing such electron movement is available than is the case for the ATP formation that is somehow coupled to this electron flow.

Photophosphorylation. Three ATP molecules are generally required for the reductive fixation of one CO_2 molecule into a carbohydrate (cf. Fig. 5.1). Such ATP is produced by *photophosphorylation*, i.e., light absorbed by the photosynthetic pigments in the lamellar membranes leads to a flow of electrons to which is coupled the phosphorylation of ADP. We will consider the energetics of this dehydration of ADP plus phosphate to yield ATP in the next chapter.

Photophosphorylation was first demonstrated in cell-free systems in 1954. A. Frenkel, working with bacterial chromatophores, and D. I. Arnon, M. B. Allen, and F. R. Whatley, using broken spinach chloroplasts, observed ATP formation in the light (see Arnon *et al.*, and Frenkel 1954). So far, very little is known at the molecular level about the enzymes involved in photophosphorylation or the actual mechanism for ATP formation. One difficulty is that the enzymes are localized in or on the lamellar membranes, and the energy transfer steps are apparently very sensitive to disruption of the solid system. Another difficulty is that none of the molecular species (ADP, ATP, and phosphate) can be readily determined quantitatively *in vivo* (see Simonis and Urbach). It has not been possible so far to monitor the interconversions of these compounds in the chloroplasts by measuring changes in spectral properties, a technique that is quite successful for studying the acceptance or donation of electrons by cytochromes and trap chl's. Furthermore, all three molecules (ATP, ADP, and phosphate) take part in many different biochemical reactions, and this greatly complicates the interpretation of the kinetic data on photophosphorylation presently available. We will reconsider ATP formation coupled to electron flow in both chloroplasts and mitochondria in the next chapter, after some of the underlying energy concepts are introduced.

Problems

5.1 A spherical spongy mesophyll cell is $40\ \mu\text{m}$ in diameter and contains 50 spherical chloroplasts $4\ \mu\text{m}$ in diameter. Assume that such cells contain 1 mg chlorophyll/g, that the cell is 90% water by weight, and that the cellular density is $1.00\ \text{g/cm}^3$. (a) What volume fraction of the cell is occupied by chloroplasts? (b) If the CO_2 fixation rate is $100\ \mu\text{moles/mg chlorophyll-hr}$, how long does it take to double the dry weight of the cell? Assume that CO_2 and H_2O are the only substances entering the cell. (c) If the ratio Chl *a*/Chl *b* is 3, what is the mean molecular weight of chlorophyll? (d) Assuming that the chlorophyll is uniformly

distributed throughout the cell, what is the maximum absorbance by one cell in the red and the blue regions? Use absorption coefficients given in Figure 5.3.

5.2 Suppose that some pigment has 8 double bonds in conjugation and has a single absorption band with a $\lambda_{\max}$ at 580 nm, which corresponds to a transition to the fourth vibrational sublevel of the excited state (i.e., the 3rd excited vibrational sublevel, cf. Fig. 4.6). A similar pigment has 10 double bonds in conjugation, which causes the lowest vibrational sublevel of the excited state to move down in energy by 0.2 eV while the lowest vibrational sublevel of the ground state moves up in energy by 0.2 eV compared with the analogous levels in the other molecule. Assume that the splitting between vibrational sublevels remains at 0.1 eV and that the most likely transition predicted by the Franck-Condon principle for this second molecule is also to the fourth vibrational sublevel. (a) What is the $\lambda_{\max}$ for fluorescence by each of the two molecules? (b) Can either or both molecules readily pass their excitation on to Chl *a* *in vivo*? (c) Can the absorption of blue light by Chl *a* lead to excitation of either of the pigments? Give your reasoning.

5.3 Let us approximate chloroplasts by short cylinders 4 μm in diameter and 2 μm thick (i.e., 2 μm along the cylinder axis) which contain 20 mM chlorophyll. The chloroplasts are exposed to 4×10^4 ergs/cm²-sec of 675 nm light parallel to the axis of the cylinder. Assume that ϵ_{675} is 0.60×10^5 liters/mole-cm for the chlorophylls. (a) What is the absorbance at 675 nm for the chlorophyll in a single chloroplast? What is the fraction of the incident light absorbed? (b) How many photons/cm²-sec of 675 nm light will be absorbed in passing through a single chloroplast? How many chlorophyll molecules participate in this absorption? (c) Assume that a photosynthetic unit has 250 chlorophyll molecules and that 0.01 sec is needed to process each excitation. How often are chlorophyll molecules excited on the average and what fraction of the absorbed photons can be processed? (d) How many molecules of oxygen are evolved per minute for 1 cm² and for each chloroplast? Assume that 8 photons are needed in order to evolve one molecule of oxygen. (e) What would be the answers to (c) for a chloroplast that is shaded by three overlying chloroplasts?

5.4 Chloroplasts corresponding to 10 nanomoles of chlorophyll/ml of solution are suspended in a cuvette with a 1 cm light path. The rate of oxygen evolution is proportional to the visible radiation up to 6×10^{14} photons absorbed/cm²-sec, which gives 10^{-7} M O₂ evolved/sec. For a very brief and intense flash of light, the O₂ evolution is 5×10^{-9} M. (a) Using the above data, how many photons are required per O₂ evolved? (b) How many chlorophyll molecules are there in a photosynthetic unit? (c) How much time is required for the processing of an excitation by a photosynthetic unit? (d) An "uncoupler" is a compound which decreases the ATP formation coupled to photosynthetic electron flow. When such a compound is added to chloroplasts incubated at a high illumination, the O₂ evolution rate is higher for the first few seconds and then becomes less than a control without the uncoupler. Explain.

5.5 Suppose that the absorbance of pea chloroplasts in a cuvette with a 1 cm light path is 0.1 at 710 nm and 1.0 at 550 nm. Assume that chlorophyll is the only species absorbing at 710 nm and that no chlorophyll absorbs at 550 nm. Suppose that no

CO₂ is fixed when either 550 nm or 710 nm light is used alone, but that both together lead to CO₂ fixation. (a) Is any ATP formation caused by the 550 nm or by the 710 nm light? (b) What type of pigments are absorbing at 550 nm? Are they isoprenoids or tetrapyrroles? (c) If equal incident photon fluxes of low intensity are simultaneously used at both 550 nm and 710 nm, what is the maximum quantum yield for CO₂ fixation for each beam? (d) The initial bleaching of P₇₀₀ at 700 nm leads to a decrease in absorbance of 0.00001 in 10⁻⁶ sec. What is the minimum number of photons per unit area absorbed by Photosystem I that could account for this? Assume that ϵ_{700} is 0.8×10^5 liters/mole-cm for the trap chl.

References

- Arnon, D. I. 1967. Photosynthetic activity of isolated chloroplasts. *Physiological Reviews* **47**: 317-358.
- Arnon, D. I., M. B. Allen, and F. R. Whatley. 1954. Photosynthesis by isolated chloroplasts. *Nature* **174**: 394-396.
- Bishop, N. I. 1971. Photosynthesis: The electron transport system of green plants. *Annual Review of Biochemistry* **40**: 197-226.
- Boardman, N. K. 1970. Physical separation of the photosynthetic photochemical systems. *Annual Review of Plant Physiology* **21**: 115-140.
- Brown, J. S. 1972. Forms of chlorophyll in vivo. *Annual Review of Plant Physiology* **23**: 73-86.
- Cheniae, G. M. 1970. Photosystem II and O₂ evolution. *Annual Review of Plant Physiology* **21**: 467-498.
- Clayton, R. K. 1965. *Molecular Physics in Photosynthesis*, Blaisdell, New York.
- Davies, D. D., J. Giovanelli, and T. Ap Rees. 1964. *Plant Biochemistry*, Blackwell, Oxford.
- Dickerson, R. E. 1972. The structure and history of an ancient protein. *Scientific American* **226**: No. 4 (April), 58-72. (Offprint No. 1245.)
- Duysens, L. M. N., J. Ames, and B. M. Kamp. 1961. Two photochemical systems in photosynthesis. *Nature* **190**: 510-511.
- Emerson, R. 1957. Dependence of yield of photosynthesis in long-wave red on wavelength and intensity of supplementary light. *Science* **125**: 746.
- Fogg, G. E. 1968. *Photosynthesis*, American Elsevier, New York.
- Fork, D. C., and J. Ames. 1969. Action spectra and energy transfer in photosynthesis. *Annual Review of Plant Physiology* **20**: 305-328.
- Frenkel, A. 1954. Light-induced phosphorylation by cell-free preparations of photosynthetic bacteria. *Journal of the American Chemical Society* **76**: 5568-5569.
- Frenkel, A. W. 1970. Multiplicity of electron transport reactions in bacterial photosynthesis. *Biological Reviews* **45**: 569-616.
- Glazer, A. N., G. Cohen-Bazire, and R. Y. Stanier. 1971. Characterization of phycoerythrin from a *Cryptomonas* sp. *Archiv für Microbiologie* **80**: 1-18.

- Goedheer, J. C. 1972. Fluorescence in relation to photosynthesis. *Annual Review of Plant Physiology* **23**: 87-112.
- Goodwin, T. W. (ed.). 1965. *Chemistry and Biochemistry of Plant Pigments*, Academic Press, London.
- Gregory, R. P. F. 1971. *Biochemistry of Photosynthesis*, Interscience (Wiley), London.
- Heath, R. I. 1973. The energy state and structures of isolated chloroplasts: The oxidative reactions involving the water-splitting step of photosynthesis. *International Review of Cytology* **34**: 49-101.
- Hill, R., and F. Bendall. 1960. Function of the two cytochrome components in chloroplasts: A working hypothesis. *Nature* **186**: 136-137.
- Hind, G., and J. M. Olson. 1968. Electron transport pathways in photosynthesis. *Annual Review of Plant Physiology* **19**: 249-282.
- Hoch, G., and R. S. Knox. 1968. Primary processes in photosynthesis, in *Photo-physiology*, Vol. III, A. C. Geise, ed., Academic Press, New York, pp. 225-251.
- Isler, O. (ed.). 1971. *Carotenoids*, Birkhäuser Verlag, Basel.
- Kamen, M. D. 1963. *Primary Processes in Photosynthesis*, Academic Press, New York.
- Ke, B. 1973. The primary acceptor of Photosystem I. *Biochimica et Biophysica Acta* **301**: 1-33.
- Kirk, J. T. O., and R. A. E. Tilney-Bassett. 1967. *The Plastids*, W. H. Freeman and Company, London and San Francisco.
- Levine, R. P. 1969. The analysis of photosynthesis using mutant strains of algae and higher plants. *Annual Review of Plant Physiology* **20**: 523-540.
- Myers, J. 1971. Enhancement studies in photosynthesis. *Annual Review of Plant Physiology* **22**: 289-312.
- Ó hEocha, C. 1971. Pigments of the red algae, *Oceanography and Marine Biology, An Annual Review* **9**: 61-82.
- Park, R. B., and J. Biggins. 1964. Quantasome: Size and composition. *Science* **144**: 1009-1011.
- Park, R. B., and P. V. Sane. 1971. Distribution of function and structure in chloroplast lamellae. *Annual Review of Plant Physiology* **22**: 395-430.
- Pauling, L. 1964. *College Chemistry*, 3rd ed., W. H. Freeman and Company, San Francisco.
- Pearlstein, R. M. (ed.). 1971. The photosynthetic unit. *Photochemistry and Photobiology* **14**: 231-473.
- Rabinowitch, E., and Govindjee. 1969. *Photosynthesis*, Wiley, New York.
- Rosowski, J. R., and B. C. Parker (eds.). 1971. *Selected Papers in Phycology*, privately printed, Department of Botany, University of Nebraska, Lincoln.
- San Pietro, A. (ed.). 1971. *Photosynthesis* (Vol. XXIII, Part A, in the series *Methods in Enzymology*), Academic Press, New York.
- Simonis, W., and W. Urbach. 1973. Photophosphorylation in vivo. *Annual Review of Plant Physiology* **24**: 89-114.
- Vernon, L. P., and G. R. Seely (eds.). 1966. *The Chlorophylls*, Academic Press, New York.

BIOENERGETICS

Throughout this text we have considered various aspects of energy in biological systems. The concept of chemical potential was introduced in Chapter 2 and then applied to the specific case of water. In Chapter 3 we used this thermodynamic approach to discuss the movement of ions. We also considered the use of energy for actively transporting some substance in an uphill direction. Chapter 4 was concerned with the absorption of light, an event which is followed by various de-excitation reactions for the excited states of the molecules. Photosynthesis (Ch. 5) involves the conversion of such electromagnetic energy into forms which are useful biologically. This last aspect, namely the production and use of various energy currencies in biological systems, is the topic of this chapter.

Two energy "currencies" produced in chloroplasts soon after the trapping of radiant energy are ATP and NADPH_2 . These substances represent the two main classes of energy storage compounds associated with the electron transfer pathways of photosynthesis and respiration. We can appreciate the importance of ATP by noting that about 0.1 mole of ATP ($\cong 50$ g) is utilized in the synthesis of 1 g dry weight ($\cong 10$ g wet weight) of many microorganisms. Since there is typically only about 10^{-5} mole of ATP per g dry weight of cells, a great turnover of ATP is necessary both to synthesize new tissue and to maintain mature cells in a state far from equilibrium (see Forrest and Walker, Payne, or Penning de Vries).

In this chapter we will first examine energy storage in terms of the chemical potential changes accompanying the conversion of a set of reactants into their products. This consideration of the Gibbs free energy allows us to determine the amount of chemical energy a given reaction can store or release. We will then evaluate the energy-carrying capacity of ATP in terms of the energetics of its formation and hydrolysis. NADPH_2 can be regarded

as possessing electrical energy, the particular amount depending on the oxidation-reduction potential of the system with which it interacts. After considering ATP and NADPH_2 as individual molecules, we will place them in their biological context, viz., as part of the bioenergetic scheme of chloroplasts and mitochondria. We will conclude with a brief comment on the overall flow of energy from the sun through the biosphere and back into space.

Gibbs free energy

Using the appropriate thermodynamic relations, we can calculate the energy changes which accompany biological reactions. Two conditions which are often met by physiological processes greatly simplify these calculations. First, most biological reactions take place at *constant temperature*, i.e., they are isothermal. Second, processes in cells or tissues generally occur at *constant pressure*. These two special conditions make the Gibbs free energy, G , a very convenient function for describing energetics in biology, since the decrease in G under these conditions equals the maximum amount of energy available for work. (See Ch. 2 for an introduction to G , and App. VII for a mathematical presentation of the Gibbs free energy.) Biological use of free energy, or "work," takes on a number of different forms, all the way from muscular movement to chemical synthesis and active transport. The difference in Gibbs free energy between two states can be used to predict the spontaneous direction for a reaction and to indicate how much energy the transition makes available for performing work. Biologists are generally interested only in such *changes* in free energy, rather than in its absolute amount, which must be defined relative to some arbitrary level (see Morowitz 1970 or Spanner).

The spontaneous direction for a reaction at constant T and P is toward a minimum of the Gibbs free energy of the system—minimum Gibbs free energy is actually achieved at equilibrium (see Fig. 6.1). In principle, a spontaneous process can always be harnessed to do work; the reversal of a spontaneous reaction requires an input of free energy (see p. 55).* As we will show below, light can be harnessed to produce a free energy source which causes the phosphorylation of ADP and the reduction of NADP, these

* This statement is a useful way of paraphrasing the second law of thermodynamics, and was first perceived by G. N. Lewis in the 1920s—see Spanner.

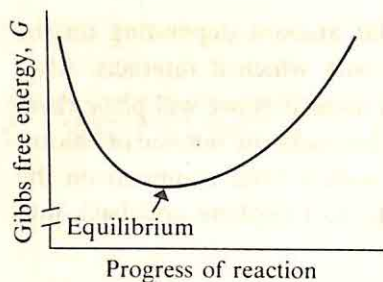


Figure 6.1

Relation between progress of a reaction and the Gibbs free energy of the system (G). When the product concentration is low (abscissa values near the origin), the reaction will spontaneously proceed in its forward direction toward lower values of G . The Gibbs free energy for a reaction attains a minimum at equilibrium; ΔG is then zero for the reaction proceeding a short distance in either direction. For high product concentrations (right side of the abscissa), ΔG to drive the reaction further in the forward direction is positive, indicating that a free energy input is then needed. When ΔG in the forward direction is positive, ΔG for the reverse reaction is negative. The absolute value of G is actually arbitrary (e.g., $G = \sum_j n_j \mu_j$, where each μ_j contains an unknown constant, μ_j^*), and so the ordinate is interrupted in the figure.

two processes being prime examples of energy-requiring reactions at the very heart of chloroplast bioenergetics.

The concept of free energy was introduced in Chapter 2 in presenting the chemical potential, μ_j . The chemical potential is actually the partial molal Gibbs free energy with respect to that species, i.e., $\mu_j = (\partial G / \partial n_j)_{T,P,E,h,n_i}$ (Eq. App. VII.9). We must therefore consider the Gibbs free energy of an entire system consisting of many components in order to define the chemical potential. In turn, we will find it convenient to regard the magnitude of G as dependent on each of the species present. An appropriate expression for the Gibbs free energy is

$$G = \sum_j n_j \mu_j \quad (6.1)$$

where n_j is the number of moles of species j in the system being considered, μ_j is the chemical potential of species j in the system (Eq. 2.4), and the summation is over all of the species present (see App. VII, where Eq. 6.1 is derived). The Gibbs free energy is expressed relative to some arbitrary zero level; the arbitrariness in the baseline for G in Equation 6.1 is a

consequence of the μ_j^* included in each μ_j (cf. Fig. 6.1). The Gibbs free energy as represented by Equation 6.1 is in a form very useful for our application of free energy relations to bioenergetics.

Chemical reactions and equilibrium constants. From the thermodynamic point of view, we are interested in the overall change in free energy for an individual reaction or perhaps sequence of reactions. Let us consider a general chemical reaction for which A and B are the reactants and C and D are the products:



where a , b , c , and d are the number of moles of the various species taking part in the reaction.

How much energy is stored (or released) when the reaction proceeds a certain extent in either direction? More specifically, what would be the change in Gibbs free energy for the reaction in Equation 6.2 proceeding in the forward direction with a moles of A and b moles of B reacting to give c moles of C and d moles of D ? For most applications, we can consider the chemical potentials of the species involved as actually constant—in other words, we are concerned with a hypothetical change in the Gibbs free energy were the reaction to take place at certain concentrations and other fixed conditions. Using Equation 6.1 ($G = \sum_j n_j \mu_j$), we can express the change

in the Gibbs free energy for such a reaction as

$$\Delta G = -a\mu_A - b\mu_B + c\mu_C + d\mu_D \quad (6.3)$$

where Δn_j is positive for a product and negative for a reactant ($\Delta G = \sum_j \Delta n_j \mu_j$ when all μ_j are constant). Equation 6.3 indicates that the free energy change for a chemical reaction can be simply the Gibbs free energy of the products minus that of the reactants.

To transform Equation 6.3 into a more useful form, we need to incorporate expressions for the chemical potentials of the various species involved. The chemical potential of species j has already been presented in Chapter 2, where μ_j was given as a linear combination of its component parts, viz., μ_j equals $\mu_j^* + RT \ln a_j + \bar{V}_j P + z_j F E + m_j g h$ (Eq. 2.4).† Substituting such

† μ_j^* is a constant, a_j the activity of species j , $\bar{V}_j$ its partial molal volume, P the pressure in excess of atmospheric, z_j its charge, F the Faraday, E the electrical potential, m_j its mass per mole, and h the vertical position in the gravitational field.

chemical potentials of A , B , C , and D into Equation 6.3, and collecting similar terms, we obtain:

$$\begin{aligned}\Delta G = & -a\mu_A^* - b\mu_B^* + c\mu_C^* + d\mu_D^* \\ & + RT(-a \ln a_A - b \ln a_B + c \ln a_C + d \ln a_D) \\ & + P(-a\bar{V}_A - b\bar{V}_B + c\bar{V}_C + d\bar{V}_D) \\ & + FE(-az_A - bz_B + cz_C + dz_D) \\ & + gh(-am_A - bm_B + cm_C + dm_D)\end{aligned}\quad (6.4)$$

Let us next simplify Equation 6.4. If the volume of the products ($c\bar{V}_C + d\bar{V}_D$) is the same as that of the reactants ($a\bar{V}_A + b\bar{V}_B$), the parenthesis multiplying P in Equation 6.4 is zero—a situation closely approached for many chemical reactions. The actual value of $P \sum_j n_j \bar{V}_j$ for biochemical reactions is usually relatively small for pressures encountered in plant cells, so we will not retain these terms here.* The algebraic sum of the terms in the second to last parenthesis in Equation 6.4 equals zero because no charge is created or destroyed by the reaction given in Equation 6.2, i.e., the total charge of the products ($cz_C + dz_D$) equals that of the reactants ($az_A + bz_B$). Since no mass is created or destroyed by the reaction, the last parenthesis is also zero. The constant terms in Equation 6.4 ($-a\mu_A^* - b\mu_B^* + c\mu_C^* + d\mu_D^*$) can be replaced by ΔG^* , a quantity which we will evaluate below.

We can now convert Equation 6.4 to a relatively simple form. Using ΔG^* , the constancies of charge and mass, and the generally valid assumption that $P(-a\bar{V}_A - b\bar{V}_B + c\bar{V}_C + d\bar{V}_D)$ is negligible, the change in Gibbs free energy for the reaction in Equation 6.2 proceeding in the forward direction becomes simply

$$\Delta G = \Delta G^* + RT \ln \frac{(a_C)^c (a_D)^d}{(a_A)^a (a_B)^b} \quad (6.5)$$

where various properties of logarithms as presented in Appendix V have been used to obtain the form indicated. Equation 6.5 is a general expression which gives the Gibbs free energy stored or released by a chemical reaction.

* Ignoring $P \sum_j n_j \bar{V}_j$ may not be valid for reactions occurring under very high pressures (e.g., deep in the ocean), especially if volume changes are suspected, such as those which can occur when two oppositely charged species react to form a neutral one—see Zimmerman.

The quantity ΔG^* in Equation 6.5 has a special value and meaning. At equilibrium, the argument of the logarithm in Equation 6.5— $[(a_C)^c (a_D)^d]/[(a_A)^a (a_B)^b]$ —is simply the equilibrium constant (K) of the reaction given by Equation 6.2. Furthermore, at constant T and P the Gibbs free energy achieves a minimum at equilibrium (Fig. 6.1), and it would not change for a conversion of reactants to products under such conditions, i.e., ΔG equals zero for a chemical reaction proceeding a short distance in either direction at equilibrium under these conditions. Equation 6.5 thus indicates that $\Delta G^* + RT \ln K$ is zero at equilibrium, and so

$$\Delta G^* = -RT \ln K \quad (6.6)$$

where K is the equilibrium constant of the reaction, i.e., K equals the equilibrium value for $[(a_C)^c (a_D)^d]/[(a_A)^a (a_B)^b]$. Hence ΔG^* depends in a rather simple fashion on the equilibrium constant of the particular reaction we are considering.

To see one of the implications of ΔG^* equaling $-RT \ln K$, let us consider that the reactants (A and B) and the products (C and D) initially all have unit activity, i.e., $[(a_C)^c (a_D)^d]/[(a_A)^a (a_B)^b]$ is one, hence the logarithm of the activity term is zero. Equation 6.5 indicates that for a moles of A plus b moles of B reacting to form c moles of C plus d moles of D under these conditions, the Gibbs free energy change, ΔG , is simply ΔG^* . If the equilibrium constant (K) for the reaction is greater than 1, ΔG^* is negative ($\Delta G^* = -RT \ln K$, Eq. 6.6), which means that in this case of unit activity of all reactants and products the reaction in the forward direction is spontaneous. For $K > 1$ we would of course expect this reaction to proceed in the forward direction, since at equilibrium the products are then favored over the reactants—in the sense that $[(a_C)^c (a_D)^d] > [(a_A)^a (a_B)^b]$ at equilibrium. On the other hand, if K is less than one, ΔG^* is positive, and such a reaction would not proceed spontaneously in the forward direction for the given initial condition of unit activity of all reactants and products.

We shall now comment on the units of ΔG and ΔG^* . G is an extensive variable (see App. VII), and it should have the units of energy. Specifically, Equation 6.1 ($G = \sum_j n_j \mu_j$) indicates that the Gibbs free energy

is the sum over all species present of the energy/mole of species j (μ_j , an intensive variable) times the number of moles of species j (n_j , an extensive variable), hence G has the dimensions of energy. On the other hand, Equation 6.5 suggests that G has the same units as RT , viz., energy/mole (a logarithm is generally considered to be dimensionless). To help us out of this dilemma, let us reconsider the conventions used in Equation 6.2

($aA + bB \rightleftharpoons cC + dD$). We usually write a chemical reaction using the smallest possible integers for a , b , c , and d , not the actual number of moles reacting. In fact, in the equations describing nearly all biochemical reactions, either or both a and c are unity, e.g., $\text{ADP} + \text{phosphate} \rightleftharpoons \text{ATP} + \text{H}_2\text{O}$. Another convention is to express the Gibbs free energy change per mole of the species upon which attention is being focused, e.g., per mole of a certain reactant ($\Delta G/a$) or per mole of a certain product ($\Delta G/c$). When a or c represents one mole, ΔG then has the same magnitude both as energy and as energy/mole. When we use actual values for ΔG or ΔG^* to describe chemical reactions, we will always indicate on what basis we are using the Gibbs free energy, e.g., " ΔG per mole of ATP formed."

What are the numerical values of ΔG^* for K s of 100 and 0.01? Since RT is 592 cal/mole at 25° and $\ln$ equals 2.303 $\log$ (App. III), an equilibrium constant of 100 corresponds to a $\Delta G^*/a$ or $\Delta G^*/c$ given by Equation 6.6 of $-(592 \text{ cal/mole})(2.303) \log(100)$, or -2730 cal per a moles of reactant A or c moles of product C , while a K of 0.01 leads to a $\Delta G^*/a$ or $\Delta G^*/c$ of $+2730 \text{ cal/mole}$. In the former case Equation 6.5 indicates that 2.73 kcal of energy/mole of the reactant or product under consideration (assuming a or c is 1 mole) would be released, while in the latter case the same amount of energy would be required to drive the reaction in the forward direction, starting with unit activity of all reactants and products.

Interconversion of chemical and electrical energy. To help understand how chemical energy can be converted into electrical energy, and vice versa, we must re-examine the properties of both chemical reactions and the movement of charged species. Let us first consider a chemical reaction such as the dissociation of sodium chloride: $\text{NaCl} \rightleftharpoons \text{Na}^+ + \text{Cl}^-$. Although two charged species are produced upon dissociation of NaCl , no overall change in the electrical components of the chemical potentials of Na^+ and Cl^- occurs. In other words, the electrical term ($z_i FE$) for Na^+ ($z_{\text{Na}} = +1$) is balanced by an opposite change in the electrical component of μ_{Cl} ($z_{\text{Cl}} = -1$). Next, let us consider the following type of reaction: $\text{Ag}_s \rightleftharpoons \text{Ag}^+ + e^-$, i.e., the dissociation of solid silver to an ion plus an electron. Again, no net change in the electrical contributions to the two chemical potentials occurs for the dissociation as written. However, the production of an electron opens up various other possibilities, since electrons can be conducted in special ways to regions where the electrical potential may be different. Such reactions, in which electrons are produced and then conducted to regions of different electrical potential, allow for a very useful interconversion of chemical and electrical energy. The electron-producing or -consuming reactions are referred to as *electrode*, or *half-cell*, reactions and occur not only

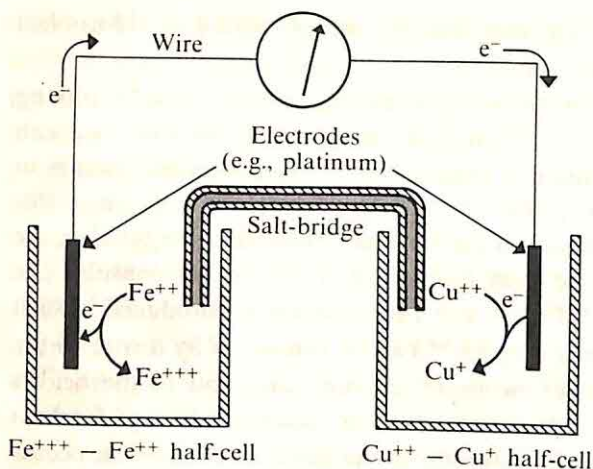
in batteries but also in the electron transfer chains located in chloroplast lamellae and mitochondrial membranes.

To elaborate on this matter of energy conversion, let us consider mixing ferrous (Fe^{++}) and cupric (Cu^{++}) ions in an aqueous solution—we can assume that the common anion is chloride. A chemical reaction occurs in which the products are ferric (Fe^{+++}) and cuprous (Cu^+) ions. Since this is a spontaneous process, there is a decrease in Gibbs free energy; also, the reaction is exothermic, since heat is evolved. Next, let us consider the electrode reaction, $\text{Fe}^{++} \rightleftharpoons \text{Fe}^{+++} + e^-$. If the electrons produced in such a half-cell initially containing only Fe^{++} can be *conducted* by a wire to the cupric ions (Fig. 6.2), another electrode reaction can occur in the beaker initially containing only Cu^{++} , viz., $\text{Cu}^{++} + e^- \rightleftharpoons \text{Cu}^+$. Except for heat evolution, the net result in the solutions is the same as that which occurs by mixing Fe^{++} and Cu^{++} . However, when the electrons move in the conductor they can be used to do various types of electrical work, e.g., powering a direct-current electric motor.

The important thing for obtaining electrical work from the two half-cell reactions is the special conducting pathway between them. If the wire from the iron to the copper electrode were immersed in the two solutions, the spontaneous flow of electrons in the wire would cause a heat evolution in the solutions. The evolved heat would be the same as that released when Fe^{++} and Cu^{++} are mixed. The other arrangement differs by having a pair of electrodes connected by a wire, with a saltbridge return path which introduces no unwanted electrical potential drops (Fig. 6.2). Such an arrangement provides a way of converting the change in Gibbs free energy of the two spontaneous half-cell reactions into electrical energy which could then be used for performing work. (See Castellan, MacInnes, Morris, or Weyer for a discussion of half-cells.)

If by some external means, e.g., by using the chemical energy in a battery, electrons are moved to a lower electrical potential ($\Delta E < 0$), then the electrical energy term ($z_j FE$) in their chemical potential is increased ($z_j = -1$ for an electron). We can use this increase in the electrical energy of the electrons to power a chemical reaction. This is done of course in electrolysis. In photosynthesis, light energy is used to move electrons toward lower electrical potentials, which sets up a spontaneous flow of electrons in the other direction. This latter, energetically downhill, spontaneous electron movement is harnessed to drive the photophosphorylation reaction ($\text{ADP} + \text{phosphate} \rightleftharpoons \text{ATP} + \text{H}_2\text{O}$) in the forward direction and thereby to store chemical energy.

Now let us consider the interconversion of chemical and electrical energy

**Figure 6.2**

Two half-cells, or redox couples, connected by a wire and a saltbridge in order to complete the electrical circuit. Electrons donated by Fe⁺⁺ to one electrode are conducted by the wire to the other electrode where they reduce Cu⁺⁺. Both electrodes (couples) are necessary before electrons can flow. A saltbridge, which completes the electrical circuit, often contains agar and KCl in order to provide a conducting solution which does not introduce large and unknown diffusion potentials at the junctions between the saltbridge and the solutions in the beakers (see App. IX, e.g., p. 468).

in more formal terms. Suppose that n moles of electrons ($z_j = -1$) are transferred from one region to another, where the electrical potential differs by ΔE (e.g., from one half-cell to another). As we discussed on p. 93, the charge carried by a mole of protons is the Faraday (F), hence the total charge moved is $-nF$ in the present case. Electrical work is expressed as the charge transported times the electrical potential difference through which it moves (ΔE). The change in the electrical energy of n moles of electrons is therefore $-nF\Delta E$. This can be converted to an equal change in Gibbs free energy (ΔG):

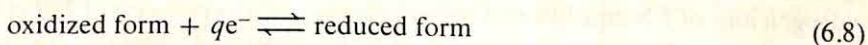
$$\Delta G = -nF\Delta E \quad (6.7)$$

According to Equation 6.7, the amount of Gibbs free energy which can be stored or released is directly proportional to the difference in electrical potential across which the electrons move. Moreover, this equation indicates

that the flow of electrons toward more positive electrical potentials ($\Delta E > 0$) corresponds to a decrease in free energy ($\Delta G < 0$), thus, this movement would be expected to proceed spontaneously, as is indeed the case. Finally, we should emphasize that two half-cells are necessary in order to get a ΔE and thus a ΔG for electron transfer (cf. Fig. 6.2). We will apply these free energy considerations to the energetics of electrons moving from molecule to molecule in the electron transfer chains of chloroplasts and mitochondria.

Redox potentials. Many organic compounds involved in photosynthesis can accept or donate electrons (cf. Table 5.1). The electrons spontaneously flow toward more positive electrical potentials, which are measured by so-called *redox potentials* in the case of the components involved with electron flow in chloroplast lamellae or mitochondrial membranes. Redox potentials are a measure of the relative chemical potential of electrons accepted or donated by a particular type of molecule. The oxidized and reduced forms of each electron transfer component can be regarded as an electrode, or half-cell. Such a half-cell can interact with the other electron-accepting and -donating molecules in the membrane, in which case the electrons spontaneously move toward the component with the higher redox potential.

We will begin by writing a chemical reaction describing a general electrode (half-cell) reaction. We can represent the acceptance or donation of electrons by some species as follows:



where q is a dimensionless parameter indicating the number of electrons transferred per molecule, and "oxidized" and "reduced" refer to different forms of the same species, e.g., NADP is an oxidized form and NADPH₂ is the corresponding reduced form (q is 2 in this case). Like any other chemical reaction, an oxidation-reduction reaction such as Equation 6.8 has a change in Gibbs free energy associated with it when the reactants are converted to products. Thus oxidation-reduction, or "redox," reactions can be described by the relative tendency of the redox system, or couple—the oxidized plus the reduced forms of the compound—to proceed in the forward direction, which for Equation 6.8 means accepting electrons.

It is more useful to describe redox reactions in terms of relative electrical potentials instead of the equivalent changes in Gibbs free energy. The electrons in Equation 6.8 come from some other redox couple, and whether or not the reaction in Equation 6.8 will proceed in the forward direction depends on the relative electrical potentials of these two couples. It is therefore convenient to assign a particular electrical potential value to a

system accepting or donating electrons, a value known as its *redox potential*. This oxidation-reduction potential can then be compared with that of another couple to predict the direction for spontaneous electron flow when the two systems are allowed to interact, viz., electrons spontaneously move toward higher redox potentials. The redox potential of species j , E_j , is defined as follows:

$$E_j = E_j^* - \frac{RT}{qF} \ln \frac{(\text{reduced}_j)}{(\text{oxidized}_j)} \quad (6.9)$$

where E_j^* is an additive constant, q is the number of electrons transferred (the same q as in Eq. 6.8), and (reduced_j) and (oxidized_j) refer to the activities of the two different redox states of species j . Equation 6.9 indicates that the oxidation-reduction potential of a particular redox couple is determined by the ratio of the reduced to the oxidized form plus an additive constant, a quantity which we will consider next.

We can measure electrical potentials when two electrodes (half-cells) are connected and pathways for electron flow are provided (see Fig. 6.2). Since the sum of the electrical potential drops (voltage changes) going completely around such a circuit is zero, we can determine the half-cell potential for a particular electrode if the potential of some standard reference electrode is known. By international agreement, the E_j^* of a hydrogen half-cell ($\frac{1}{2}\text{H}_{2\text{gas}} \rightleftharpoons \text{H}^+ + \text{e}^-$) is arbitrarily set equal to zero for an activity of hydrogen ions of 1 N equilibrated with hydrogen gas at a pressure of 760 mm Hg, i.e., $E_{\text{H}}^* = 0$. Fixing the zero level of the electrical potential for the hydrogen half-cell removes the arbitrary nature of redox potentials for all half-cells, since the redox potential for any species can then be determined relative to that of the hydrogen electrode. We will replace E_j^* in Equation 6.9 by $E_j^{*,\text{H}}$ in order to emphasize the convention of referring electrode potentials to the standard hydrogen electrode.

According to Equation 6.9, the larger the $(\text{reduced}_j)/(\text{oxidized}_j)$, the more negative the redox potential becomes. Since electrons are negatively charged, an algebraically lower E_j corresponds to higher energies for the electrons. Thus, the further Equation 6.8 is driven in the forward direction, the more energy will be required to reduce species j . Likewise, the larger the $(\text{reduced}_j)/(\text{oxidized}_j)$, the higher will be the electrical energy of the electrons which the reduced form of that couple can then donate. When (reduced_j) equals (oxidized_j) , E_j equals $E_j^{*,\text{H}}$ by Equation 6.9. This $E_j^{*,\text{H}}$ is commonly referred to as the *midpoint redox potential* (cf. the values given in Table 5.1 for the $E_j^{*,\text{H}}$ of some components involved with electron transfer in chloroplasts). For certain purposes, knowledge of the midpoint redox po-

tentials only may be sufficient, as we will show later in this chapter (see Lehninger; Morris; or White *et al.* for more details on redox reactions).

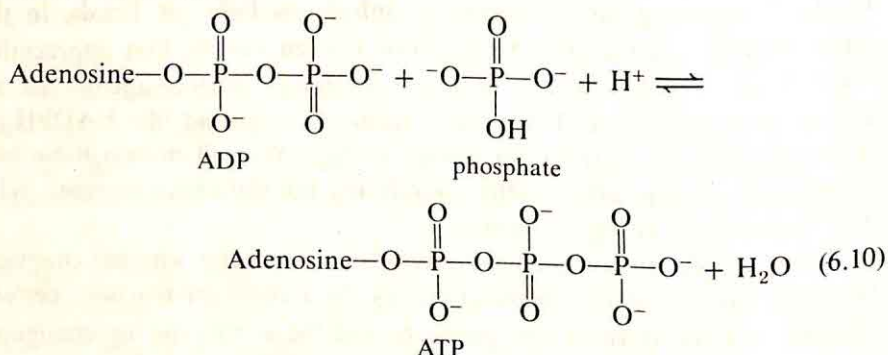
Biological energy currencies

In photosynthesis photons are captured, creating an electron flow leading to both the production of NADPH_2 and a coupled process whereby ATP is formed. Light energy is converted into chemical energy in the formation of a phospho-anhydride (ATP) in an aqueous environment. It is converted into electrical energy by providing a reduced compound (NADPH_2) under oxidizing conditions. ATP and NADPH_2 are the two energy storage compounds, or “currencies,” that we will consider in this section. Both can occur as ions, both can readily diffuse around in the cell or organelle, and both can carry appreciable amounts of energy under biological conditions. In addition to its use in processes such as active transport and muscle contraction, the chemical energy stored in ATP is also used in certain anabolic reactions involving the formation of anhydrous links, or bonds, in the aqueous milieu of the cell. Atmospheric oxygen insures that appreciable amounts of oxygen—which is of course a strong oxidizing agent—will be present in most biological systems; a reduced compound like NADPH_2 is thus an important currency for energy storage. We will discuss these two compounds in turn, after briefly considering the difference between ATP and NADPH_2 as energy currencies.

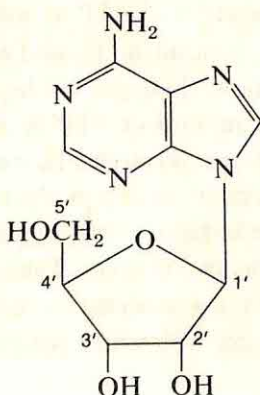
Redox couples are assigned a relative electrical energy, whereas chemical reactions have a specific chemical energy. In a chemical reaction, certain reactants are transformed into products, and the accompanying change in Gibbs free energy can be calculated. This change in chemical energy need not depend on any other system. For instance, if the concentrations—strictly speaking, the chemical activities—of ADP, phosphate, and ATP as well as certain other conditions (e.g., temperature, Mg^{++} concentration, and ionic strength) are the same in different parts of a plant, then the Gibbs free energy released upon the hydrolysis of a certain amount of ATP to ADP and phosphate will be the same in each of the different locations. However, an oxidation-reduction couple must donate electrons to, or accept electrons from another redox system, and the change in electrical energy depends on the difference in the redox potential between the two couples. Thus, the amount of electrical energy released when NADPH_2 is oxidized to NADP depends on the redox potential of the particular couple with which NADPH_2 interacts.

ATP, structure and reactions. To help us understand the bioenergetics of chloroplasts and mitochondria we need to know how much energy is stored in ATP, that is, the difference between its chemical potential and that of the reactants (ADP and phosphate) used in its formation. We must then look for reactions which have a large enough free energy decrease in order to drive the ATP production reaction in the energetically uphill direction; this will lead us to a consideration of the energetics of electron flow in organelles—topics we will discuss in the next two sections. Our immediate concern here is with the following: (1) the chemical reaction describing ATP formation (2) the associated change in Gibbs free energy for that reaction and (3) the implications of the substantial amount of energy storage in ATP.

ADP, ATP, and phosphate all can occur in a number of different charge states in aqueous solutions. Moreover, all three compounds can interact with other species present, notably magnesium and calcium ions. Thus, many different chemical reactions can be used for describing ATP formation. A predominant reaction occurring near neutral pH in the absence of divalent cations is as follows:



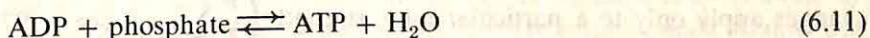
where adenosine is adenine esterified to the 1' position of the sugar ribose, i.e.,



The attachment of adenosine to the phosphates in ADP and ATP—and in NADP as well as in FAD, see page 277—is by means of an ester linkage with the hydroxymethyl group on the 5' position of the ribose moiety (see Fig. 6.3).

Equation 6.10 indicates a number of features of ATP production. For instance, the formation of ATP from ADP plus phosphate is a dehydration, while the reversal of Equation 6.10, in which the phospho-anhydride is split with the incorporation of water, is known as ATP *hydrolysis*. Since Equation 6.10 contains H^+ , the equilibrium constant depends on pH, $-\log(a_{H^+})$. Moreover, the fractions of ADP, phosphate, and ATP in various states of ionization depend on the pH. Near pH 7 about half of the ADP molecules are doubly charged and half are triply charged, the latter form being indicated in Equation 6.10.* Likewise, ATP at pH 7 is about equally distributed between the forms with charges of -3 and -4 . Due to their negative charges in aqueous solutions, both ADP and ATP can readily bind positive ions, especially divalent cations such as magnesium or calcium. A chelate is formed, such that Mg^{++} or Ca^{++} is electrostatically held between two negatively charged oxygen atoms on the same molecule (cf. the many $-O^-$ occurring on the chemical structures indicated in Eq. 6.10). Also, inorganic phosphate can electrostatically interact with magnesium and other divalent cations, further increasing the number of complexed forms of ADP, ATP, and phosphate that are possible.

The activities (or concentrations) of a species in all of its various ionization states and complexed forms are generally summed to obtain the total activity (or concentration) of that species. The number of relations and equilibrium constants needed to describe a reaction like ATP formation is then reduced to one, i.e., a separate equilibrium constant is not needed for every possible combination of ionization states and complexed forms of all the reactants and products. Using this convention, we can replace Equation 6.10 and many others like it, which also describe ATP formation, by the following general reaction for the phosphorylation of ADP:



We will return to ATP formation as represented by Equation 6.11 after briefly commenting on two important conventions used in biochemistry. First, most equilibrium constants for biochemical reactions are defined at

* For simplicity, we are ignoring the charge due to the extra proton bound to a purine nitrogen of adenosine, which gives that part of the molecule a single positive charge at pH 7.

a specific pH, usually pH 7 ($a_{H^+} = 10^{-7}$ M). At constant pH the activity of H^+ does not change. Thus, H^+ need not be included as a reactant or product in the expression for the change in Gibbs free energy (Eq. 6.5). In other words, the effect of H^+ in relations such as Equation 6.10 is actually incorporated into the equilibrium constant, which itself generally depends on pH. Second, biological reactions such as ATP formation usually take place in aqueous solutions where the concentration of water does not change appreciably. (The concentrations of other possible reactants and products are much, much less than that of water.) Hence, the (a_{H_2O}) term coming from relations like Equation 6.11 is also usually incorporated into the equilibrium constant. We can illustrate these points concerning a_{H^+} and a_{H_2O} by specifically considering ATP formation as described by Equation 6.10. For the reaction as written, the equilibrium constant (K) is equal to $[(a_{ATP})(a_{H_2O})]/[(a_{ADP})(a_{\text{phosphate}})(a_{H^+})]$, hence $(a_{ATP})/[(a_{ADP})(a_{\text{phosphate}})]$ equals $(a_{H^+}) K/(a_{H_2O})$. For a dilute aqueous solution a_{H_2O} is essentially constant during the reaction; therefore, $(a_{H^+}) K/(a_{H_2O})$ —which is conveniently called the equilibrium constant in biochemistry—has a fixed value at a given pH, e.g., at pH 7, $(a_{H^+}) K/(a_{H_2O}) = (10^{-7} \text{ M}) K/(a_{H_2O}) = K_{\text{pH } 7}$.

Next, we will specifically consider the equilibrium constant for ATP formation under biological conditions. Using the above conventions for H^+ and H_2O , the observed equilibrium constant for Equation 6.11 at pH 7 ($K_{\text{pH } 7}$) is

$$K_{\text{pH } 7} = \frac{[ATP]}{[ADP][\text{phosphate}]} \cong 4.2 \times 10^{-7} \text{ M}^{-1} \text{ at } 25^\circ \quad (6.12)$$

where the total concentration of each species involved is indicated in brackets, i.e., it was experimentally more convenient to measure $K_{\text{pH } 7}$ using concentrations (indicated here always by brackets) instead of activities (designated always by parentheses).

When activities of ions ($a_j = \gamma_j c_j$, Eq. 2.5) are replaced by concentrations (c_j), the resulting equations for equilibrium constants or free energy changes apply only to a particular ionic strength $\left(\frac{1}{2} \sum_j c_j z_j^2\right)$, see p. 97, because the activity coefficients of ions (γ_j) can markedly depend on ionic strength (cf. Eq. 3.3). Actually, the value for $K_{\text{pH } 7}$ given in Equation 6.12 is suitable only for ionic strengths close to 0.2 M, which is a reasonable estimate for the ionic strengths generally occurring *in vivo*. The magnitude of the equilibrium constant for ATP formation also depends on the concentration of magnesium ions. The value of $K_{\text{pH } 7}$ indicated in Equation 6.12 is appropriate for 1 to 10 mM Mg^{++} (see Alberty), a concentration range occurring in many plant cells.

Gibbs free energy change for ATP formation. The energetics of a reaction like ATP formation is summarized by its Gibbs free energy change, ΔG . For a general chemical reaction, Equation 6.5 indicates that ΔG equals $\Delta G^* + RT \ln \{[(a_C)^c (a_D)^d]/[(a_A)^a (a_B)^b]\}$, where ΔG^* is $-RT \ln K$ (Eq. 6.6). For the present case, the reactant A is ADP, B is phosphate, the product C is ATP, and the equilibrium constant is given by Equation 6.12. Therefore, our sought-after free energy relationship describing ATP formation is

$$\begin{aligned}\Delta G &= -RT \ln (K_{\text{pH } 7}) + RT \ln \frac{[\text{ATP}]}{[\text{ADP}][\text{phosphate}]} \quad (6.13) \\ &\cong 8.7 + 1.36 \log \frac{[\text{ATP}]}{[\text{ADP}][\text{phosphate}]} \text{ kcal/mole of ATP at pH } 7 \\ &\hspace{15em} \text{and } 25^\circ\end{aligned}$$

where $\ln$ equals $2.303 \log$, $2.303 RT$ is 1.364 kcal/mole at 25° (App. III), and $-(1.364 \text{ kcal/mole}) \log (4.2 \times 10^{-7})$ is 8.7 kcal/mole .

It is apparent from Equation 6.13 that ATP usually does not tend to form spontaneously because the Gibbs free energy change for the reaction is generally quite positive. In fact, the actual amount of energy required for the phosphorylation of ADP is rather large compared with the free energy changes for most biochemical reactions. Stated another way, much energy can be stored by converting ADP plus phosphate to ATP. It is interesting to note that although ATP in an aqueous solution is *thermodynamically* unstable, or "metastable," in the sense that its hydrolysis can release a considerable amount of Gibbs free energy, it still can last for a long enough time in cells (i.e., *kinetics*) in order to be an important energy currency. In particular, ATP is generally not hydrolyzed very rapidly unless the appropriate proteins necessary for its use in anabolic reactions or other processes are present.

We will now estimate the changes in Gibbs free energy which might be expected for photophosphorylation under physiological conditions. For purposes of calculation, we will assume that in unilluminated chloroplasts the concentrations of ADP and phosphate are initially each 10 mM and ATP is 1 mM —actual concentrations in the dark are probably somewhat less than these. $[\text{ATP}]/\{[\text{ADP}][\text{phosphate}]\}$ is then $(10^{-3} \text{ M})/[(10^{-2} \text{ M})(10^{-2} \text{ M})]$, or 10 M^{-1} before illumination begins. From Equation 6.13, the free energy change required to form ATP is $8.7 + 1.36 \log (10)$, or 10.1 kcal/mole of ATP. The change in Gibbs free energy required is positive, indicating that energy must be supplied to power photophosphorylation. Moreover, the energy necessary depends in a predictable way on the concentrations of the reactants and the product. After a certain period of time in the light, ATP

may increase to 10 mM with a concomitant decrease in ADP and phosphate to 1 mM. The ratio $[ATP]/\{[ADP][phosphate]\}$ would then become $(10^{-2})/[(10^{-3})(10^{-3})]$, or 10^4 M^{-1} . The free energy required for photophosphorylation under these conditions is $8.7 + 1.36 \log(10^4)$, or 14.1 kcal/mole of ATP. Consequently, the further photophosphorylation goes to completion, the greater is the energy required to form more ATP.

The high energy of ATP relative to ADP plus phosphate for usual physiological concentrations is not the property of a single bond but of the local configuration in the ATP molecule, a point we can appreciate by considering the phosphorus atoms in ADP, ATP, and phosphate. Phosphorus is in group V of the third period of the periodic table and has 5 electrons in its outermost shell. It can enter into a total of 5 bonds with 4 oxygen atoms, the bonding to one O being a double bond (cf. structures in Eq. 6.10). In inorganic phosphate all 4 bonds are equivalent, so that 4 different structures for phosphate exist in resonance with each other. The terminal P of ADP has only 3 resonating forms, since one of the oxygens is connected to a second phosphorus atom and does not assume a double bond configuration (see Eq. 6.10). When inorganic phosphate is attached to this terminal P of ADP to form ATP, a resonating form is lost both from the ADP and from the phosphate. Since configurations having more resonating structures are in general more probable or stable (lower in energy), energy must be supplied to form ATP from ADP plus phosphate with an accompanying loss of 2 resonating forms.

Let us next examine quantitatively some of the ways in which ATP can be used as a free energy currency. Each of our four examples will relate to a different variable term in the chemical potential ($\mu_j = \mu_j^* + RT \ln a_j + \bar{V}_j P + z_j F E + m_j g h$, Eq. 2.4). To transfer a mole of a neutral compound against a 10-fold increase in activity requires $2.303 RT$, or 1.364 kcal of Gibbs free energy at 25° , while it takes 2.7 kcal for a 100-fold difference and 4.1 kcal for a thousandfold increase in activity. To move a monovalent cation from one side of a membrane to the other, where it has the same concentration but the electrical potential is 0.1 v higher, requires 2.3 kcal/mole transported ($F = 23.06 \text{ kcal/mole-v}$, App. II). ATP usually supplies at least 10 kcal/mole hydrolyzed and can act as the Gibbs free energy source for the active transport of solutes across membranes toward regions of higher chemical potential. ATP is also the free energy currency for the contraction of muscles. The ATP-driven contraction of the muscles surrounding the left ventricle of the human heart can increase the blood pressure within it by 0.2 bar. This increases the chemical potential of the water in the blood (i.e., the $\bar{V}_w P$ term) which causes it to flow out to the aorta and then to the rest of the circulatory system toward lower hydrostatic

pressures. Pressure-driven flow is quite an efficient way to move fluids, e.g., it takes only 0.005 kcal of Gibbs free energy to increase the pressure of 1 liter of water by 0.2 bar. As an example of gravitational work which can be mediated by ATP, the increase in Gibbs free energy as a 50 kg person climbs up 100 m is 12 kcal.

NADP-NADPH₂ redox couple. Another class of energy storage compounds consists of redox couples such as NADP-NADPH₂. The reduced form, NADPH₂, is produced by noncyclic electron flow in chloroplasts. Photosynthesis in bacteria makes use of a different redox system, NAD-NADH₂. The reduced member of this latter couple causes an electron flow in mitochondria and an associated formation of ATP (see "Mitochondrial Bioenergetics," p. 288). NAD is nicotinamide adenine dinucleotide and differs from NADP in not having a phosphate esterified to the 2' hydroxy group of the ribose in the adenosine part of the molecule (see Fig. 6.3).

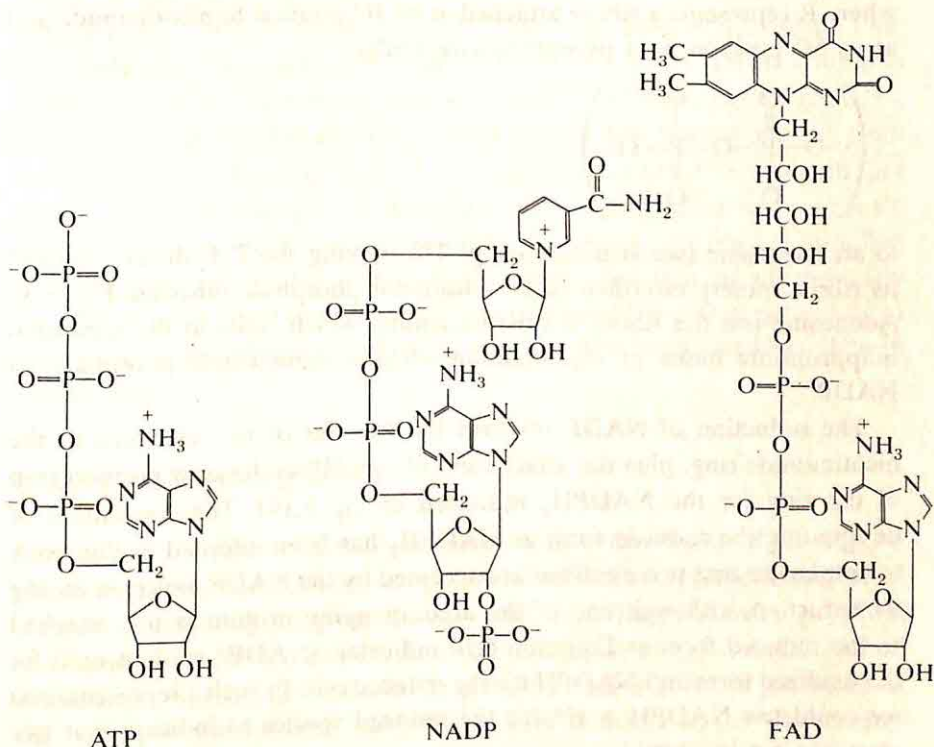
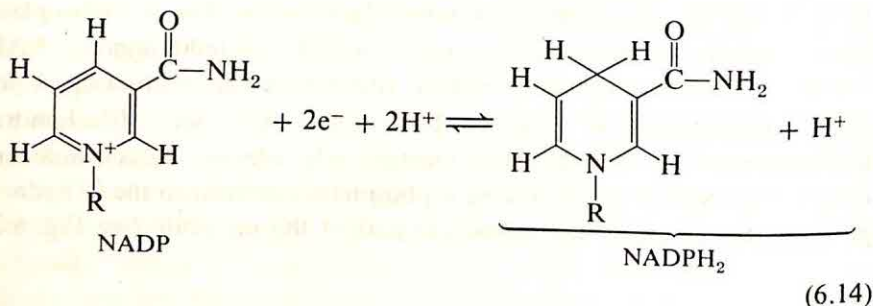


Figure 6.3

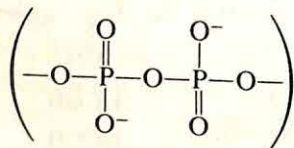
Structure of three molecules important in bioenergetics. The dissociations and bindings of protons indicated in the figure are appropriate near pH 7. Note the similarity between the molecules.

Our present discussion will focus on the NADP-NADPH₂ couple, but the same arguments and also the same midpoint redox potential apply to the NAD-NADH₂ system (see Lehninger; Morris; or White *et al.*).

The reduction of a molecule of NADP involves the acceptance of two electrons by it. Only the nicotinamide portion (illustrated in Eq. 6.14) of NADP is involved in accepting the electrons. The actual half-cell reaction describing this reduction is



where R represents a ribose attached at its 1C position to nicotinamide and at its 5C position by a pyrophosphate bridge



to an adenosine (see structure on p. 272) having the 2' hydroxy group of its ribose moiety esterified to an additional phosphate (also see Fig. 6.3). Adenosine less the ribose is called adenine, which leads to the somewhat inappropriate name of nicotinamide adenine dinucleotide phosphate, or NADP.

The reduction of NADP involves the transfer of two electrons to the nicotinamide ring, plus the attachment of one H⁺ to the *para* position (top of the ring for the NADPH₂ indicated in Eq. 6.14). The convention of designating the reduced form as NADPH₂ has been adopted in this book to emphasize that two electrons are accepted by the NADP molecule during its reduction, although one of the accompanying protons is not attached to the reduced form as Equation 6.14 indicates. (NADP⁺ is often used for the oxidized form and NADPH for the reduced one. In such a representation we could use NADPH + H⁺ for the reduced species to indicate that two electrons are involved.)

A particular half-cell reaction such as Equation 6.14 can accept or donate electrons. We can quantitatively describe this by the oxidation-reduction

(redox) potential for that reaction, as expressed by Equation 6.9 $\{E_j = E_j^{*,H} - (RT/qF) \ln [(reduced_j)/(oxidized_j)]\}$. We will use $(NADPH_2)$ to represent the activity of all of the various ionization states and complexed forms of the reduced nicotinamide adenine dinucleotide phosphate, while $(NADP)$ has an analogous meaning for the oxidized species of the $NADP-NADPH_2$ couple. For redox reactions of biological interest the midpoint (standard) redox potential is generally determined at pH 7. By using Equation 6.9, in which the number (q) of electrons transferred per molecule reduced is 2, we can express the oxidation-reduction potential of the $NADP-NADPH_2$ couple (Eq. 6.14) as follows:

$$\begin{aligned} E_{NADP-NADPH_2} &= E_{pH7}^{*,H} - \frac{RT}{2F} \ln \frac{(NADPH_2)}{(NADP)} \\ &= -320 - 29.6 \log \frac{(NADPH_2)}{(NADP)} \text{ mv at } 25^\circ \text{ and pH } 7 \end{aligned} \quad (6.15)$$

where $\ln$ has been replaced by $2.303 \log$ and the numerical value for $2.303 RT/F$ of 59.2 mv at 25° (App. III) has been used in the second line. The midpoint redox potential, $E_{pH7}^{*,H}$, for the $NADP-NADPH_2$ couple is -320 mv (Table 5.1), a value achieved when $(NADPH_2)$ equals $(NADP)$.

To determine whether electrons spontaneously flow toward or away from the $NADP-NADPH_2$ couple, we must compare its redox potential with that of some other redox couple. As we indicated above, electrons spontaneously flow toward more positive redox potentials, whereas energy must be supplied to move electrons in the energetically uphill direction of algebraically decreasing redox potential. We will next examine the redox potentials of the various redox couples involved in electron flow in chloroplasts, then in mitochondria.

Chloroplast bioenergetics

In Chapter 5 various molecules involved with electron transfer in chloroplasts were introduced, together with a consideration of the sequence of electron flow among components. Now that the concept of redox potential has been presented we will resume our discussion of electron transfer in chloroplasts. Specifically, we will compare the midpoint redox potentials of the various redox systems, not only to help understand the direction of spontaneous electron flow, but also to see whether a sufficient ΔE is available between certain pairs of redox couples in order to drive the ATP formation reaction in the forward direction.

Redox couples. Although the ratio of reduced to oxidized forms of species j affects its redox potential $\{E_j = E_j^{*,H} - (RT/qF) \ln [(\text{reduced}_j)/(\text{oxidized}_j)]\}$, Eq. 6.9, the actual activities of the two forms are usually not known for biological studies *in vivo*. Moreover, the value of the local pH (which can affect $E_j^{*,H}$) is also generally unknown. Consequently, we can usually only compare midpoint (standard) redox potentials determined at pH 7 to predict the direction for spontaneous electron flow in lamellar membranes of chloroplasts, and we will follow such an approach here. In general, free energy is required in order to transfer electrons to a compound with a more negative *midpoint* redox potential, while the reverse process can go on spontaneously.

The absorption of a photon can markedly affect the redox properties of a pigment molecule. An excited molecule such as trap chl* has an electron in an antibonding orbital (cf. Ch. 4). It takes less energy to remove such an electron from the excited molecule than when that molecule is in its ground state. Thus, the electronically excited molecule is a better reducing agent than the ground state, inasmuch as it has a considerably more negative redox potential. Once the electron is removed, the oxidized molecule (trap chl⁺) becomes a very good electron acceptor. This electron acceptance, trap chl⁺ + $D \rightarrow$ trap chl + D^+ (Eq. 5.6), actually involves the ground state of the chlorophyll molecule, which has a rather positive redox potential (see Table 5.1 and Fig. 6.4). The electronic state of trap chl which donates an electron is therefore an excited state with a negative redox potential, while the ground state with its rather positive redox potential can readily accept an electron. In short, the absorption of light energy transforms trap chl's from couples with positive redox potentials to couples with negative ones. In this section we will estimate the redox potential spans at each of the two photosystems in chloroplasts and then diagram the overall pattern of photosynthetic electron flow.

Following light absorption by Chl *a* or an accessory pigment in Photosystem II, the excitation migrates to the trap chl where an electron transfer reaction takes place (trap chl* + $A \rightarrow$ trap chl⁺ + A^- , Eq. 5.5). The electron which is removed from trap chl* of Photosystem II is replaced by one coming from water, which results in oxygen evolution as described by Equation 5.8. (For energetic considerations, we can write Eq. 5.8 as $H_2O \rightleftharpoons \frac{1}{2}O_2 + 2H^+$.) The water-oxygen system represents a half-cell reaction with a midpoint redox potential of 0.82 v at 25°, pH 7, and an oxygen pressure of 760 mm Hg (cf. Fig. 6.4). Water oxidation and the accompanying oxygen evolution (Eq. 5.8) follow spontaneously after the photochemistry at the reaction center of Photosystem II has led to a trap chl⁺. Thus, the

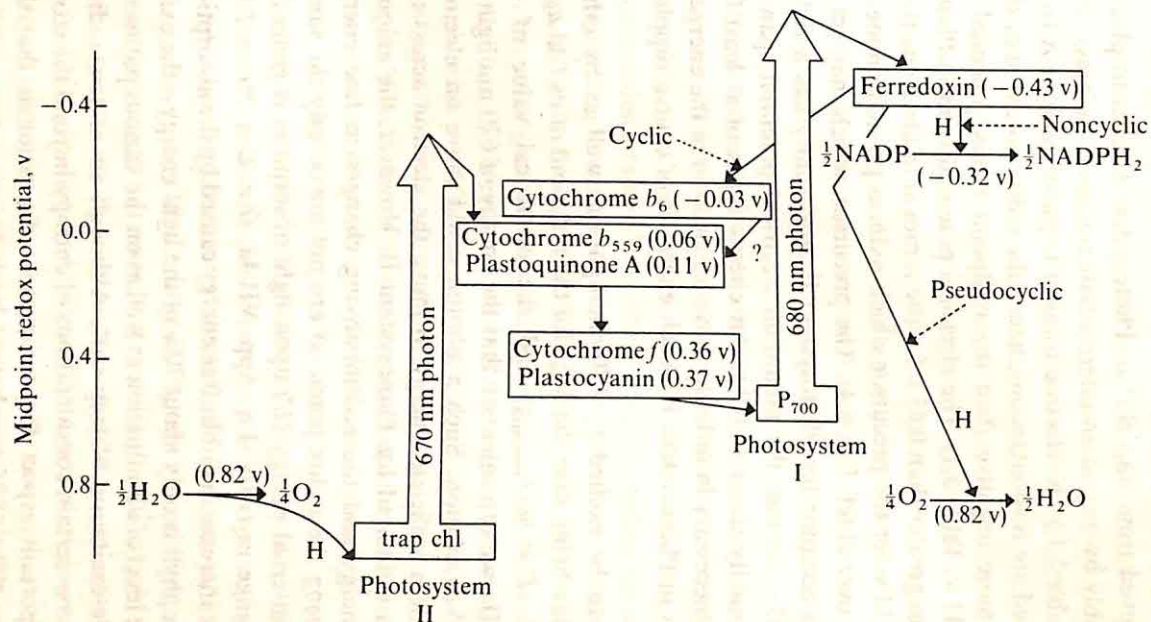


Figure 6.4

Energy aspects of photosynthetic electron flow. The lengths of the arrows emanating from the trap chl's of Photosystems I and II represent the increase in chemical potential of the electrons that occurs upon absorption of red light at the λ_{max} 's of the main Chl a 's in the respective photosystems. The diagram also shows the various midpoint redox potentials of the couples involved (data from Table 5.1) and the three types of electron flow mediated by ferredoxin.

required oxidant for water, i.e., trap chl⁺ in Photosystem II or some intermediate oxidized by it, must have a redox potential more positive than 0.82 v in order for the electron to move energetically downhill from water to the trap chl⁺ in the reaction center.

The electron removed from trap chl* of Photosystem II goes to plastoquinone A, presumably by way of an intermediate (or intermediates). (See references cited in Table 5.1.) The electron transfer to plastoquinone A from the proposed intermediate is spontaneous, hence the redox potential of this precursor must be more negative than the midpoint redox potential of plastoquinone A (0.11 v, Table 5.1). The electrical potential span in Photosystem II is thus from greater than 0.82 v for the removal of electrons from water to less than 0.11 v for the precursor which reduces plastoquinone A, or more than 0.71 v overall (cf. Fig. 6.4). The precursor, which may act as the primary electron acceptor for Photosystem II, apparently has a redox potential near -0.05 v, so that the minimum electrical potential span in Photosystem II is actually closer to 0.9 v. An energy input of at least 0.7 to 0.9 ev is therefore necessary in order to move an electron in the energetically uphill direction in Photosystem II—such energy is of course supplied by a light quantum.

Photosystem II can be excited by 670 nm light—as well as by other wavelengths, this value being near the $\lambda_{\max}$ for the red band of its Chl *a*₆₇₀. From Equation 4.2 ($E = hc/\lambda_{\text{vacuum}}$) and the numerical value of hc (1240 ev-nm, App. III), we can calculate that the energy of 670 nm light is (1240)/(670), or 1.85 ev/photon. Such a photon could move an electron across 1.85 v, more than sufficient energy to move the electron across the redox potential span estimated for Photosystem II. However, the amount of absorbed light energy and the accompanying changes in free energy available for decreasing the redox potential are not necessarily the same, i.e., the increase in internal energy (U) upon light absorption is generally not equal to the change in G (cf. Eq. App. VII.4a, $G = U + PV - TS$). The magnitude of the increase in Gibbs free energy caused by the absorption of a photon by chlorophyll is only about 70% of the light energy—the exact value depends on the level of illumination as well as on the various pathways competing for the de-excitation of trap chl*. Although an understanding of this situation requires certain considerations of entropy beyond the scope of this text, the important aspect for our present discussion is that the absorption of 670 nm light (1.85 ev/photon) leads to an increase of free energy of about (1.85 ev)(0.7), or 1.3 ev (see Knox as well as Ross and Calvin). This is sufficient to move an electron across the redox potential span of Photosystem II, which we estimated as somewhat more than 0.7 to 0.9 v.

We can make a similar analysis of the energetics for Photosystem I, where the trap chl is P_{700} . The known redox potential span across which electrons are moved (see Table 5.1) is from the midpoint redox potential of 0.43 v for the P_{700} - P_{700}^+ couple to the -0.43 v of ferredoxin (the latter is a quite negative standard redox potential). Since an intermediate with a midpoint redox potential even more negative than -0.43 v may intervene before ferredoxin and spontaneously reduce it, 0.86 v is an estimate of the *minimum* redox potential span for Photosystem I. (If we had used actual existing redox potentials instead of midpoint redox potentials, the minimum span calculated for Photosystem I would still be about 0.9 v.) A photon of red light at the $\lambda_{\max}$ of Chl a_{680} in Photosystem I has an energy of 1.82 ev (Table 4.1) of which about 1.3 ev is available to increase the free energy. This is ample to move the electron from P_{700} to ferredoxin and to allow extra energy for a possible intermediate with a redox potential more negative than that of ferredoxin.

From ferredoxin to the next component in the noncyclic electron flow sequence, NADP, electrons go from -0.43 v to -0.32 v (midpoint redox potentials of the couples), or a ΔE of +0.11 v (cf. Fig. 6.4). Moving toward higher redox potentials is energetically downhill for electrons, so that this step leading to the reduction of the pyridine nucleotide follows spontaneously from the reduced ferredoxin—the enzyme ferredoxin-NADP reductase catalyzes this downhill process.

In noncyclic electron flow, two electrons originating in the water-oxygen couple with a midpoint redox potential of 0.82 v are moved to the redox level of -0.32 v for the reduction of one molecule of the NADP-NADPH₂ couple. Since a midpoint redox potential of -0.32 v is more negative than most encountered in biology, NADPH₂ can spontaneously reduce most other redox systems; reduced pyridine nucleotides are therefore an important energy currency. Moving electrons from 0.82 v to -0.32 v requires a considerable expenditure of free energy, which helps to explain why light, with its relatively large amount of energy (cf. Table 4.1), is needed. We can calculate the actual free energy change for the overall process using Equation 6.7 ($\Delta G = -nF\Delta E$), where ΔE is (-0.32 v) - (0.82 v), or -1.14 v, n is 2 moles, and F is 23.06 kcal/mole-v (App. II). Hence, ΔG equals -(2 moles)(23.06 kcal/mole-v)(-1.14 v), or 52.6 kcal for the overall movement of two moles of electrons along the pathway for noncyclic electron flow—a process which leads to the reduction of one mole of NADP.

The incorporation of CO₂ into a carbohydrate during photosynthesis requires 3 ATP's and 2 NADPH₂'s (cf. Fig. 5.1). Using these energy currencies, CO₂ fixation can proceed in the absence of light, and so the steps of the reductive pentose cycle are often referred to as the "dark reactions"

of photosynthesis. The hydrolysis of ATP in chloroplasts can release about 12 kcal/mole (p. 275) and we have just stated that 53 kcal are required to reduce a mole of NADP using electrons coming from water—this is actually the Gibbs free energy change between standard states, since midpoint redox potentials were used in our calculations. The increase in Gibbs free energy per mole of CO_2 incorporated into a carbohydrate during photosynthesis is 114 kcal (p. 216). Using these numbers, we can estimate the efficiency for free energy storage by the dark reactions. Dividing the energy stored per mole of CO_2 fixed, 114 kcal, by the energy input [(3 moles ATP) (12 kcal/mole ATP) + (2 moles NADPH_2)(53 kcal/mole NADPH_2)], we find that the efficiency is $(114/142)(100)$ or 80%! Evolution has indeed led to an extremely efficient set of steps for the dark reactions of photosynthesis.

Figure 6.4 incorporates the midpoint redox potentials of the various components involved with photosynthetic electron transfer discussed in both Chapter 5 (and included in Table 5.1) and here. The direction for spontaneous electron flow to higher midpoint redox potentials is downward (toward the bottom of Fig. 6.4), while the absorption of light quanta with their relatively large energies corresponds to moving electrons vertically upward to higher energy. The role played by ferredoxin as the crossroads for cyclic, noncyclic, or pseudocyclic electron flow is also illustrated in Figure 6.4.

Coupling of ATP formation to electron flow. We argued above that electrical and chemical energies are interconvertible. Let us now apply this to reactions in chloroplasts. We might ask, for example, what difference in electrical potential or redox potential would be sufficient to supply the energy necessary to cause ADP plus phosphate to react to form ATP? The minimum change in redox potential necessary to power such formation of ATP can be calculated from Equation 6.7, $\Delta G = -nF\Delta E$, where F is 23.06 kcal/mole-v (App. II). The amount of Gibbs free energy (ΔG) required to phosphorylate a mole of ADP is generally from 10 to 14 kcal. Thus, the ΔG for the redox reaction must be at least as negative as -10 to -14 kcal. When one electron is transferred— $n = 1$ mole in Equation 6.7—this corresponds to an electrical potential change ΔE of $\Delta G/(-nF)$, or $(-10 \text{ to } -14 \text{ kcal})/[(-1 \text{ mole})(23.06 \text{ kcal/mole-v})]$, which is 0.43 to 0.61 v. If two electrons were transported per ATP formed, an electrical potential span of half this amount, namely 0.22 to 0.30 v, would be necessary.

We will next focus our attention on the site for coupling photosynthetic electron flow to ATP formation. The midpoint redox potentials in Figure 6.4 indicate the ΔE s available. First, let us consider the difference in standard redox potentials between plastoquinone A (0.11 v) and Cyt f (0.36 v). The redox potential change of 0.25 v does not represent enough energy to cause

ATP formation if the free energy decrease of a single mole of electrons is used per mole of ATP, since our above calculation indicates that 0.43 to 0.61 v would then be necessary. However, the 10 to 14 kcal needed for the phosphorylation of one mole of ADP can be supplied by two moles of electrons moving across an electrical potential span of 0.22 to 0.30 v. Thus, the span of 0.25 v between the midpoint redox potentials of plastoquinone A and Cyt *f* is apparently sufficient for ATP formation, if a pair of electrons traversing the electron transfer pathway between Photosystems II and I is used. All we can say at this stage is that such a site for photophosphorylation is thermodynamically possible.

Numerous experimental results indicate that 2 ATP's are formed for each pair of electrons moving from water to NADP (see Forti *et al.*, as well as Schwartz). Thus, two coupling sites, each using a pair of electrons per ATP, may be associated with the two photosystems plus the electron transfer pathway between Photosystems II and I. In terms of the components listed in Table 5.1 and diagrammed in Figure 6.4, the only site between plastoquinone A (midpoint redox potential of 0.11 v) or Cyt b_{559} (0.06 v) and P_{700} (0.43 v) with sufficient energy to form ATP is the coupling site using a pair of electrons which we mentioned in the previous paragraph. On the other hand, each photon absorbed by Photosystem II can lead to an increase in free energy of 1.3 ev (p. 282), of which about 0.7 to 0.9 ev is accounted for by the difference in midpoint redox potential between the water-oxygen couple (0.82 v) and the precursor (approximately -0.05 v) which reduces plastoquinone A (0.11 v). Hence, 0.4 v may still be available in Photosystem II to drive an ATP formation reaction in the forward direction (recall that an electrical potential span of 0.2 to 0.3 v is sufficient to form ATP if a pair of electrons is used). There is some evidence that a coupling site for ATP formation may exist between the water oxidation step and the trap chl⁺ of Photosystem II, although the molecular details of such a coupling site have not been elucidated (see Crofts *et al.*).

A matter related to the number of coupling sites is the relative amounts of ATP produced and NADP reduced in chloroplasts. Three moles of ATP and two of NADPH₂ are needed per mole of CO₂ photosynthetically fixed. This applies to the majority of plants, referred to as "C-3" plants since CO₂ is incorporated into ribulose-1,5-diphosphate to yield two 3-phosphoglyceric acids, a *three carbon* compound. Five ATP's are actually required per CO₂ fixed in chloroplasts from mesophyll cells of the "C-4" plants, while three are needed in the bundle-sheath cells of such plants. (We will briefly discuss C-4 plants in the next chapter; see p. 336.) If only one coupling site for ATP formation exists for Photosystems II and I, then 8 photons—4 absorbed

by each system—will give 2 NADPH₂'s and 2 ATP's (cf. Fig. 5.12). How would the third ATP necessary for CO₂ fixation in C-3 plants be produced? One hypothesis is that cyclic or perhaps pseudocyclic electron flow leads to the other ATP. (As we indicated in Ch. 5, no reduction of NADP accompanies either one of these types of electron flow.) This possibility would require 9 or 10 photons to produce the 3 ATP's plus 2 NADPH₂'s used per CO₂ fixed, a point which remains experimentally unresolved. However, two ATP's most likely are produced per pair of electrons moving in the noncyclic pathway, in which case 4 photons absorbed by each photosystem can lead to 4 ATP's and 2 NADPH₂'s. Consequently, 8 may well be the actual minimum number of photons needed to evolve one molecule of oxygen and fix one of carbon dioxide.

Coupling mechanisms. Another unresolved question in chloroplast bioenergetics is the mechanism for coupling electron flow to ATP formation, two hypotheses for which are currently being considered (see Crofts *et al.*; Forti *et al.*; Sanadi; Schwartz; White *et al.*; and Witt). In one, a so-called *chemical* hypothesis, the intermediates intervening between electron flow in the chloroplast lamellae and the joining of ADP and phosphate coupled to this are believed to be specific components, presumably proteins. The flow of electrons to certain compounds could conceivably cause changes in the electronic configuration of the involved coupling molecules, a condition ultimately leading to the formation of ATP.

In the other hypothesis, the so-called *chemi-osmotic* hypothesis put forward by P. Mitchell in the 1960s, it is proposed that the photosynthetic electron flow actively transports protons across the lamellar membranes. The energy for the uphill H⁺ movement is purportedly derived from electron transfers at the same coupling sites we mentioned above, e.g., the one lying between plastoquinone A and Cyt *f*. The overall movement of each electron in the noncyclic pathway apparently leads to the transport of two protons across the membranes (see Schwartz). This transport creates a difference in chemical potential of the hydrogen ions across the membranes; in turn, the $\Delta\mu_{H^+}$ is believed to drive the ATP formation reaction in the forward direction. Since the chemiosmotic hypothesis readily lends itself to a thermodynamic treatment, we will quantitatively examine its bioenergetics.

We begin by noting that the difference in chemical potential across a membrane for any ionic species can be calculated from Equation 3.26, $\mu_j^i - \mu_j^o = z_j F (E_M - E_{N_j})$. Moreover, we can relate the Nernst potential of hydrogen ions or protons ($E_{N_{H^+}}$) to the pH. By Equation 3.5 [$E_{N_j} = (RT/z_j F) \ln (a_j^o/a_j^i)$], $FE_{N_{H^+}}$ equals $RT \ln (a_{H^+}^o/a_{H^+}^i)$, or $2.303 RT (\log a_{H^+}^o - \log a_{H^+}^i)$, which becomes simply $2.303 RT (\text{pH}^i - \text{pH}^o)$ by the definition of pH, $-\log a_{H^+}$. Thus, using Equations 3.5 and 3.26, we can

express the difference in chemical potential of hydrogen ions across a membrane as

$$\begin{aligned}\mu_{\text{H}^+}^i - \mu_{\text{H}^+}^o &= FE_M - 2.303 RT (\text{pH}^i - \text{pH}^o) \\ &= 0.023 E_M + 1.36 (\text{pH}^o - \text{pH}^i) \text{ kcal/mole at } 25^\circ\end{aligned}\quad (6.16)$$

where F is 0.02306 kcal/mole-mv (App. II), $2.303 RT$ is 1.364 kcal/mole at 25° (App. III), and E_M in the second line is expressed in millivolts.

According to the chemi-osmotic hypothesis of Mitchell, the ATP formation reaction is driven in the forward direction by the difference in the chemical potential of protons across the lamellar membranes. Two protons may move from inside a thylakoid* out across the lamellar membranes into the chloroplast stroma for each ATP formed. We can calculate the free energy available from such proton movement by using Equation 6.16. Suppose that E_M is 0 mv and $\text{pH}^o - \text{pH}^i$ is 4; $\mu_{\text{H}^+}^i - \mu_{\text{H}^+}^o$ is then $(0.023 \text{ kcal/mole-mv})(0 \text{ mv}) + (1.36 \text{ kcal/mole})(4)$, or 5.4 kcal/mole. Two moles of protons moving down such a free energy gradient could release 10.8 kcal, which is often sufficient energy for the formation of one mole of ATP (cf. Eq. 6.13).

There is remarkable agreement between the above prediction on the ΔpH leading to ATP formation and the results of A. T. Jagendorf and others (see Jagendorf in San Pietro). When chloroplast lamellae are equilibrated with a solution at pH 4—in which case pH^i presumably attains a value near 4—and are then rapidly transferred to a medium containing ADP and phosphate with a pH^o of 8 or above, the lamellae are capable of driving the ATP formation reaction in the forward direction *in the dark*. When ΔpH is 3 or less, essentially no ATP can be formed by such chloroplast lamellae. If the pH of the bathing solution is gradually increased from 4 to 8 in the dark (over a period of tens of seconds), the protons “leak” out across the lamellar membranes, ΔpH is always considerably less than 4, and no ATP is formed.

The lamellar systems of chloroplasts studied *in vitro* are usually quite permeable to ions, so that E_M is probably close to zero for them. On the other hand, there is some evidence that chloroplast lamellae are not as permeable in the cell and therefore E_M may be substantial across the thylakoids *in vivo* (see Forti *et al.*, as well as Witt). If E_M were 150 mv (positive inside the thylakoid) and $\text{pH}^o - \text{pH}^i$ were 2, $\mu_{\text{H}^+}^i - \mu_{\text{H}^+}^o$ would be $(0.023 \text{ kcal/mole-mv})(150 \text{ mv}) + (1.36 \text{ kcal/mole})(2)$, or 6.3 kcal/mole by Equation 6.16. Two moles of protons moving across such an electrical

* A flattened sac formed from a pair of membranes, cf. Figure 1.9.

potential drop and pH increase could release nearly 13 kcal, which is generally sufficient energy to form one mole of ATP.

Another prediction of the chemi-osmotic hypothesis is that the drop in μ_{H^+} across lamellar membranes in the dark would normally not be large enough to lead to ATP formation by chloroplasts. When the organelles are illuminated, electron flow commences, which somehow causes $\mu_{H^+}^i$ within the lamellae to increase relative to the chemical potential of protons in the stroma. Thus, we would expect a delay before $\mu_{H^+}^i - \mu_{H^+}^o$ given by Equation 6.16 is sufficient to lead to ATP formation. Indeed, a lag of a fraction of a second to seconds is often observed before photophosphorylation commences, and the lag can be decreased by increasing the illumination level (see Schwartz). We can also speculate that a gradient in the chemical potential of hydrogen ions will affect the movements of other species. For instance, an energetically downhill movement of H^+ could be coupled to the uphill movement of another ion such as K^+ . Such exchange processes, for which there is considerable evidence at the lamellar level, might even occur at the organelle level and lead to the light-induced chloroplast flattening mentioned on page 76.

As we noted in the last chapter, a great deal remains to be learned about the details of photophosphorylation. Much of the current research uses compounds that block or inhibit electron flow at specific locations or that uncouple ATP formation from electron flow (see Izawa and Good, as well as other articles in San Pietro). As yet, however, we have not reached a final decision on the actual mechanism of coupling between electron flow and ATP production. Moreover, it is not understood at the molecular level how two electrons or two protons can lead to the formation of only one ATP molecule.

Mitochondrial bioenergetics

The activities of chloroplasts and mitochondria are related in a number of ways, as is clear from Figure 6.5. For instance, the O_2 evolved by photosynthesis can be consumed during respiration, while the fate of CO_2 is just the opposite in the two processes. Moreover, ATP formation is coupled to electron flow in both organelles; in mitochondria the electron flow is from a reduced pyridine nucleotide to the oxygen-water half-cell, while in chloroplasts it is in the opposite direction (see Fig. 6.5). From a few to many thousands of mitochondria occur in a given plant cell, their frequency tending to be somewhat less in cells where chloroplasts are abundant. In photosynthetic tissue at night, and at all times in the nongreen parts of the plant, oxidative phosphorylation in mitochondria is the predominant supplier of ATP for the cells.

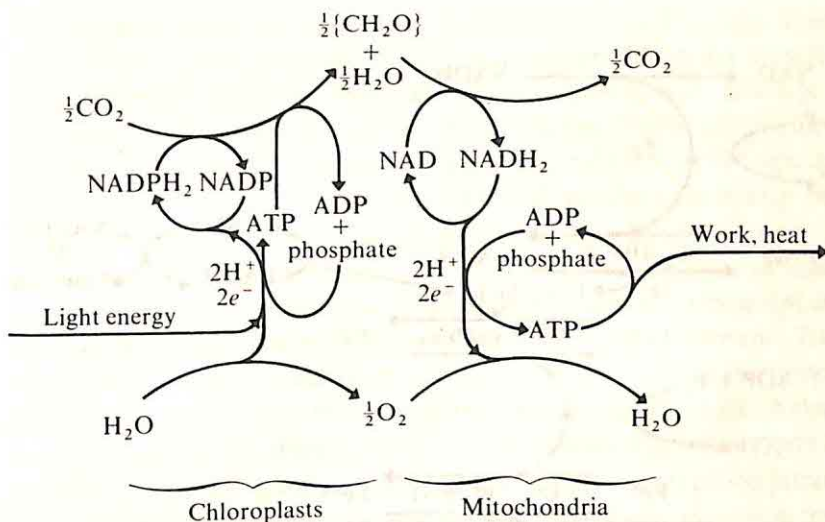
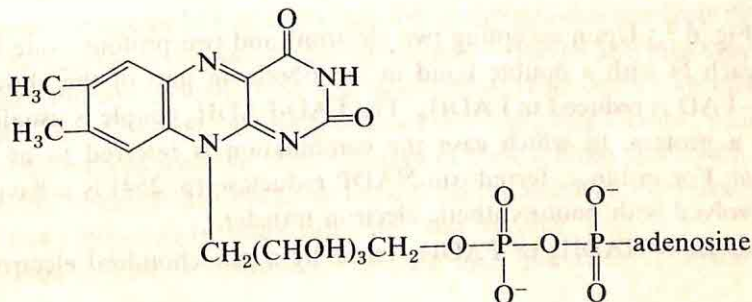


Figure 6.5

Schematic representation of the interrelationship between components involved in chloroplast and mitochondrial bioenergetics.

Electron flow components—redox potentials. As with chloroplasts, many compounds in mitochondria can accept or donate electrons. The principal components for mitochondrial electron transfer are indicated in Figure 6.6, where the spontaneous electron flow to higher redox potentials is toward the bottom of the figure. As for photosynthetic electron flow, only a relatively few types of compounds are involved in electron transfer in mitochondria, viz., pyridine nucleotides, flavoproteins containing flavin adenine dinucleotide, quinones, cytochromes, and the water-oxygen couple.

The reduced compounds which introduce electrons into the mitochondrial electron transfer chain are NADH_2 and FADH_2 (cf. Fig. 6.6). FAD is flavin adenine dinucleotide, and consists of riboflavin (vitamin B_2) bound by a pyrophosphate bridge to adenosine:



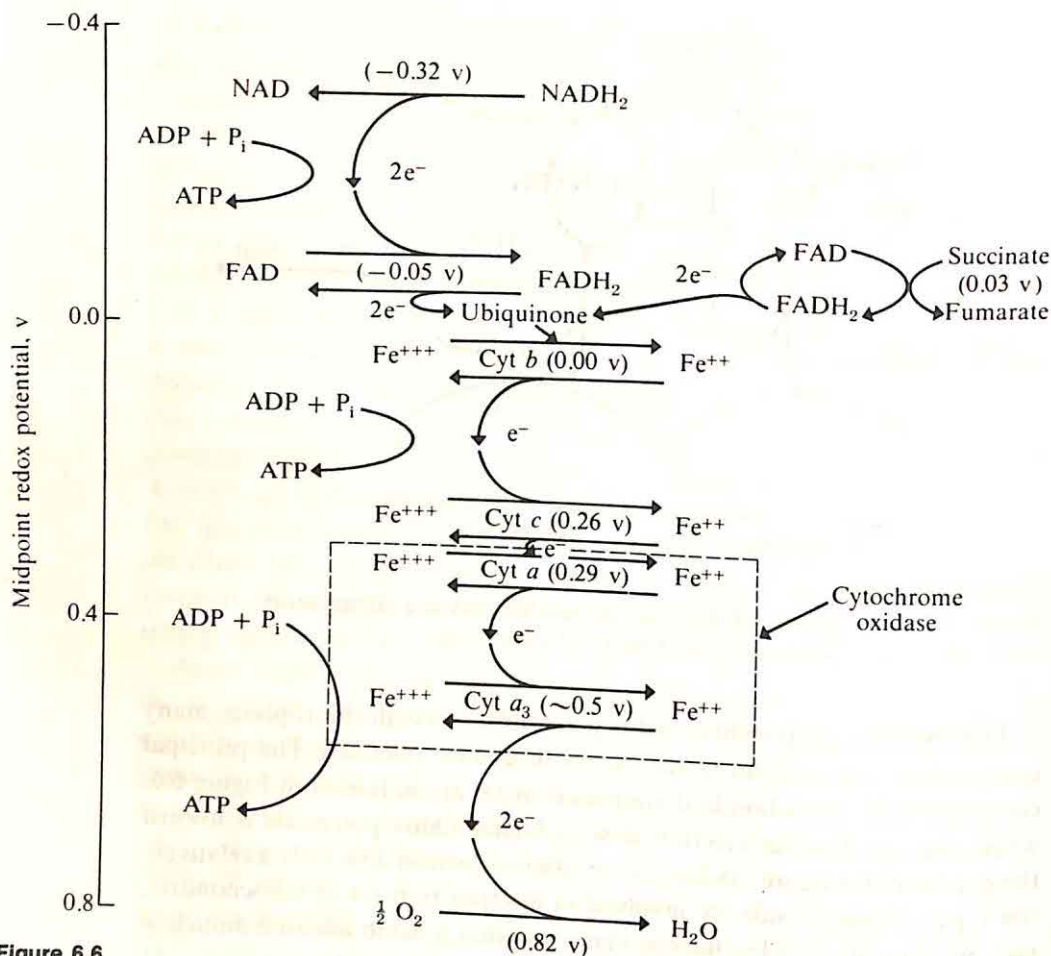


Figure 6.6

Components of the mitochondrial electron transport chain with midpoint redox potentials in parentheses. Also indicated are possible coupling sites where the movement of two moles of electrons toward higher redox potentials can supply sufficient Gibbs free energy to form one mole of ATP. (See Ikuma; Lehninger; and White *et al.*)

(Also see Fig. 6.3.) Upon accepting two electrons and two protons—one H going to each N with a double bond in the riboflavin part of the above structure—FAD is reduced to FADH₂. The FAD-FADH₂ couple is usually bound to a protein, in which case the combination is referred to as a *flavoprotein*. For instance, ferredoxin-NADP reductase (p. 254) is a flavo-protein involved with photosynthetic electron transfer.

Electrons from NADH₂ or FADH₂ move by a mitochondrial electron

transfer chain to O_2 , which is thus reduced to H_2O (see Fig. 6.6). Important members of the mitochondrial electron transfer chain, in order of increasing midpoint redox potential (indicated in parentheses), are: NAD-NADH₂ (-0.32 v), FAD-FADH₂ (-0.22 to -0.03 v, depending on the protein), Cyt *b* (0.00 v), Cyt *c* (0.26 v), Cyt *a* (0.29 v), Cyt *a*₃, which is in the cytochrome oxidase complex (~ 0.5 v), and O_2 - H_2O (0.82 v). The cytochrome oxidase complex contains 2 to 6 Cyt *a* molecules, or subunits, plus copper on an equimolar basis with the heme. The acceptance or donation of electrons by this Cu^{++} - Cu^+ couple in the cytochrome oxidase complex is apparently involved with mitochondrial electron transfer (see Hayaishi and Wainio). Mitochondria contain Coenzyme Q, or ubiquinone, which may intervene in electron transfer between the flavoproteins and Cyt *b* (Fig. 6.6), or electrons may go from FADH₂ directly to Cyt *b* (see Ikuma). The ubiquinone found in most plant and animal mitochondria differs from plastoquinone A (p. 251) by having 2 methoxy groups in place of the methyl groups on the ring, and 10 instead of 9 isoprene units in the side chain. A *c*-type cytochrome, referred to as Cyt *c*₁ in animal mitochondria, may intervene just before Cyt *c*, while there is evidence for a *b*-type cytochrome in plant mitochondria which is involved with an electron transfer that bypasses cytochrome oxidase on the way to oxygen. Cytochromes *a*, *b*, and *c* are in roughly equal amounts in mitochondria (the ratios vary somewhat with plant species—see Ikuma), while flavoproteins are about four times and pyridine nucleotides approximately twenty times more abundant than individual cytochromes. Likewise, in chloroplasts the pyridine nucleotides are much more abundant than the cytochromes (see Table 5.1).

Oxidative phosphorylation. ATP formation coupled to electron flow in mitochondria is generally called *oxidative phosphorylation*. Since electron flow involves reduction as well as oxidation, more appropriate names would be “respiratory phosphorylation” or “respiratory-chain phosphorylation,” terms which are also more consistent with photophosphorylation for the ATP formation occurring in photosynthesis. As with photophosphorylation, the mechanism of oxidative phosphorylation is not yet understood in molecular terms. Processes like phosphorylation accompanying electron flow appear to be intimately connected with membrane structure, and thus they are much more difficult to study than the biochemical reactions which take place in solutions. However, certain proteinaceous “coupling factors” which appear to be involved with the terminal steps of the coupling process for oxidative phosphorylation have been isolated from the inner membranes (cristae) of animal mitochondria (see van Dam and Meyer).

Due to lack of information on the local pH and the actual chemical

activities of the species involved, we do not know the existing redox potentials of the various mitochondrial oxidation-reduction components *in vivo*. Nevertheless, we can estimate from the midpoint redox potentials the electrical energy available between various redox couples to help indicate which electron transitions might be associated with ATP formation, the topic we turn to next.

Experiments with isolated mitochondria have shown that the number of ATP's produced per pair of electrons used to reduce oxygen depends on the particular compound which introduces the electrons into the electron transfer pathway. For instance, when a pair of electrons moves from NADH_2 to oxygen, 3 ATP molecules can be produced (see Fig. 6.6). Certain substrates such as succinate lead directly to a reduction of a flavoprotein without first reducing NAD. The oxidation of the FADH_2 reduced by succinate—which occurs on a different flavoprotein than the FAD involved with NADH_2 oxidation—leads to the phosphorylation of only 2 ADP's per pair of electrons moving along the mitochondrial electron transfer chain (cf. Fig. 6.6). This suggests that a coupling site for ATP formation may exist between NADH_2 and the appropriate FADH_2 (Fig. 6.6). From the NAD- NADH_2 couple to the FAD- FADH_2 couple with which it interacts, the difference in midpoint redox potentials is about 0.27 v. Assuming a free energy requirement of 10 to 14 kcal to form a mole of ATP, a drop in redox potential of 0.43 to 0.61 v is necessary if the electrical energy of one mole of electrons is to supply the Gibbs free energy required to form one mole of ATP; an 0.22 to 0.30 v change in electrical potential would be necessary to produce one ATP by a pair of electrons moving toward higher redox potentials (p. 284). Since about 0.27 v is available, we are forced to conclude that one ATP can be formed only if a *pair* of electrons moves between the two couples. Although enough energy for such oxidative phosphorylation may be available for electrons moving from NADH_2 to FAD, the matter remains uncertain, both because the existing redox potentials are not precisely known *in vivo* and the actual ΔG for ATP formation is not known in mitochondria.

Electrons from the reduced flavoproteins go to Cyt *b*, probably by way of ubiquinone, and then to the subsequent members of the electron transfer chain (Fig. 6.6). Energetically speaking, another coupling site for ATP formation may be between Cyt *b* (midpoint redox potential 0.00 v) and Cyt *c* (0.26 v), where about 0.26 v is available. Using Equation 6.7 ($\Delta G = -nF\Delta E$), we can calculate the change in free energy if two moles of electrons move across 0.26 v as $-(2 \text{ moles})(23.06 \text{ kcal/mole-v})(0.26 \text{ v})$, or -12 kcal, which is probably sufficient energy to form one mole of ATP in mitochondria. Thus, a coupling site for ATP formation may exist between

cytochromes *b* and *c*, as is indicated in Figure 6.6. The third site for oxidative phosphorylation is associated with the cytochrome oxidase complex, about which relatively little is certain. Since the redox potential of Cyt *a*₃ is not accurately known *in vivo*, we cannot closely estimate the free energy available between the various couples interacting with cytochrome oxidase, and thus the associated site where electron flow is coupled to ATP formation is indicated in a noncommittal way in Figure 6.6.

As with chloroplasts, many questions remain unanswered concerning electron flow and the coupled ATP formation in mitochondria. The first part of the mitochondrial electron transfer chain has a number of two-electron carriers (NAD, FAD, and ubiquinone) that must interact with the cytochromes (one-electron carriers). Do the two electrons from NAD move in sequence to the same Cyt *b* or to two different Cyt *b*'s acting in parallel? Do two electrons come from one Cyt *a*₃ to reduce an oxygen atom to its state in water? In fact, the reduction of O₂ may involve 1, 2, or 4 electrons coming from 4, 2, or 1 cytochrome, respectively. The molecular details of the coupling of electron flow to ATP formation in mitochondria are also uncertain. As with photophosphorylation in chloroplasts, both a chemical coupling hypothesis and a chemi-osmotic one involving changes in the chemical potential of hydrogen ions have been proposed to explain the relation between electron flow and the accompanying ATP formation in mitochondria. Although there have been a number of predictive successes using the chemi-osmotic hypothesis (such as the interplay of $\Delta\mu_{H^+}$, $\Delta\mu_{K^+}$, and the ΔG for ATP formation), the matter still remains unsettled (see Azzone and Massari; Palmer and Hall; Sanadi, and Schwartz).

Energy flow in the biosphere

The stage is now set to consider the overall flow of energy from the sun, through the biosphere, and back into space. In Chapter 4 we indicated that the sun leads to a radiation input of 2.00 cal/cm²-min into the earth's atmosphere (p. 171). Some of the radiant energy is trapped in the free energy increases which are needed to form ATP and NADPH₂ in chloroplasts (pp. 279–288). In turn, these energy currencies lead to the reductive fixation of CO₂ into a carbohydrate in photosynthesis (Fig. 5.1). In the same photosynthetic cells, in other plant cells, and in animal cells, the carbohydrates formed during photosynthesis can serve as the free energy source for respiration, which leads to the generation of ATP by oxidative phosphorylation (p. 291).

When used as a fuel in respiration, the carbohydrate glucose is first broken down into two molecules of pyruvate in the cell cytoplasm. Pyruvate enters the mitochondria and is eventually oxidized to CO_2 and H_2O . One mole of glucose thereby leads to the formation of a total of 38 moles of ATP (see Lehninger or White *et al.*). As we noted on p. 216, the Gibbs free energy released by the complete oxidation of a mole of glucose is 686 kcal, while about 10 kcal may be required for the phosphorylation of one mole of ADP in mitochondria. Hence, the efficiency of the many-faceted conversion of Gibbs free energy from glucose to ATP is $[(38 \text{ moles ATP})(10 \text{ kcal/mole of ATP})/(686 \text{ kcal})](100)$ or 55%, indicating that the Gibbs free energy in glucose can be rather efficiently mobilized to produce ATP. Such ATP can then be used by the cells to actively transport ions, to synthesize proteins, and to provide for growth and maintenance in still other ways. We can readily appreciate that if free energy were not constantly supplied to their cells, plants and animals would drift toward equilibrium and thus die.

One of the consequences of the flux of energy through the biosphere is the formation of rather complex and improbable molecules such as proteins and nucleic acids. Such compounds represent a considerably greater amount of Gibbs free energy than an equilibrium mixture containing the same relative amounts of the various atoms. (Equilibrium corresponds of course to a minimum in Gibbs free energy, cf. Fig. 6.1.) For instance, the non-aqueous components of cells have an average of about 0.27 ev more Gibbs free energy per atom than the same atoms at equilibrium (see Morowitz 1968). The Boltzmann energy distribution (Eq. 3.21) predicts that at equilibrium the fraction of atoms with energy in excess of E is equal to $e^{-E/kT}$ [k is 8.617×10^{-5} ev/molecule (or atom in this case)-°K, App. II]. For 25° and an E of 0.27 ev, $e^{-E/kT}$ is $e^{-(0.27 \text{ ev/atom})/(8.617 \times 10^{-5} \text{ ev/atom-°K}) (298^\circ \text{K})}$, or $e^{-10.5}$, which equals 0.000027. At equilibrium, therefore, only a very small fraction of the atoms would have an extra energy of 0.27 ev or more at 25°, although this is the *average* enrichment in Gibbs free energy per atom of the nonaqueous components in cells. It is the flux of energy from the sun through plants and animals (cf. Fig. 6.5) which leads to such an energy enrichment in the molecules and, furthermore, ensures that biological systems will be maintained in a state far from equilibrium, an essential feature of life.

Incident light—Stefan-Boltzmann law. Let us now reconsider some properties of radiating bodies. In Chapter 4 we indicated that the distribution of radiant energy per unit wavelength interval was proportional to $\lambda^{-4}/(e^{hc/\lambda kT} - 1)$ where T is the temperature of the radiation source (p. 173). This form of Planck's radiation distribution formula applies to an object

which radiates maximally, a so-called blackbody. When blackbody radiation is integrated over all wavelengths, we are then able to determine the maximum amount of energy which can be radiated by an object. Appropriate integration of Planck's radiation distribution formula leads to the following expression:

$$\text{maximum energy radiated per unit area} = \sigma T^4 \quad (6.17)$$

where σ is a constant. Although Equation 6.17 can be derived from quantum-physical considerations developed by Planck in 1900, in fact it was first proposed in the latter part of the 19th century. In 1879 J. Stefan empirically determined that the maximum radiation was proportional to the fourth power of the absolute temperature, while in 1885 L. Boltzmann interpreted this in terms of classical physics. The coefficient of proportionality (σ) was deduced from experimental measurements then available. It has become known as the Stefan-Boltzmann constant, and equals $8.130 \times 10^{-11} \text{ cal cm}^{-2} \text{ min}^{-1} \text{ }^\circ\text{K}^{-4}$ (App. II). In the case where the object does not radiate as a blackbody, the radiation emitted per unit area equals $e\sigma T^4$, where e is the emissivity (cf. p. 349). Emissivity depends on the surface material of the radiating body and achieves its maximum value of unity for a blackbody.

We will now estimate the amount of energy radiated per unit area of the sun's surface and how much of this is annually incident on the earth's atmosphere. Using Equation 6.17 and the effective surface temperature of the sun, about 5800°K (p. 173), the energy radiated is $(8.130 \times 10^{-11} \text{ cal cm}^{-2} \text{ min}^{-1} \text{ }^\circ\text{K}^{-4})(5800^\circ\text{K})^4$, or $0.92 \times 10^5 \text{ cal/cm}^2\text{-min}$. The entire output of the sun is about $3 \times 10^{33} \text{ cal/yr}$. Of this extremely large amount of solar radiation, about $2.00 \text{ cal/cm}^2\text{-min}$ is incident on the earth's atmosphere (p. 171). A year contains 525,600 min, and the projected area of the earth is $1.276 \times 10^{18} \text{ cm}^2$. Hence, the annual energy input into the earth from the sun is $(2.00 \text{ cal/cm}^2\text{-min})(1.276 \times 10^{18} \text{ cm}^2)(5.256 \times 10^5 \text{ min/yr})$, or $1.34 \times 10^{24} \text{ cal/yr}$.

The radiant energy input into the earth's atmosphere must be balanced by an equal energy output or the earth's temperature would tend to change. We can use Equation 6.17 to calculate the maximum amount of energy reradiated from the earth. The average temperature of the earth's surface is about 287°K , hence the upper limit on the amount of energy reradiated into outer space is $(8.130 \times 10^{-11})(287)^4$, or $0.552 \text{ cal/cm}^2\text{-min}$. This thermal radiation goes out from the earth's *total* surface area ($4\pi r^2$), in contrast to solar input on the *projected* area of the earth (πr^2). Using our figure of $0.552 \text{ cal/cm}^2\text{-min}$, the maximum amount of energy lost from the earth by radiation would be $(0.552)(4)(1.276 \times 10^{18})(5.256 \times 10^5)$, or $1.48 \times 10^{24} \text{ cal/yr}$.

Somewhat less than this is lost, since the earth's surface does not radiate at the maximum rate (its emissivity is less than unity). Moreover, not all of the incoming radiation actually reaches the earth's surface, since some sunlight is reflected by the clouds. In fact, the total amount of energy reradiated and reflected by the earth and its atmosphere is essentially equal to the solar input, 1.34×10^{24} cal/yr. Otherwise, there would be a compensatory change in the earth's temperature in order that the net energy balance would become zero.

Absorbed light and photosynthetic efficiency. Only a rather small fraction of the sun's energy incident on the earth's atmosphere each year is actually absorbed by photosynthetic pigments, and only a small fraction of the latter absorbed energy is stored as chemical energy of the photosynthetic products (see Gates, Nobel, Ryther, and Woodwell). Specifically, of the 1.34×10^{24} cal annually incident on the earth's atmosphere approximately 4% is absorbed by chlorophyll or other photosynthetic pigments. The radiant energy input into this part of the biosphere is thus about $(1.34 \times 10^{24})(0.04)$, or 5.4×10^{22} cal/yr. How much of this energy is annually stored in photosynthetic products? As we indicated in Chapter 5, a net of approximately 5.0×10^{16} g of carbon are annually fixed by photosynthesis (this corresponds to 13×10^{16} g of carbohydrate/yr). For each mole of CO_2 (12 g carbon) incorporated into carbohydrate, approximately 114 kcal of Gibbs free energy are stored. The total amount of energy stored each year by photosynthesis is thus $(5.0 \times 10^{16} \text{ g/yr})(1 \text{ mole}/12 \text{ g})(114,000 \text{ cal/mole})$, or about 4.8×10^{20} cal/yr. Hence, only about 1% of the radiant energy absorbed by photosynthetic pigments (approximately 5.4×10^{22} cal/yr) ends up stored by plant cells.

There are many different ways in which to represent the efficiency of photosynthesis. We can express it on the basis of the solar radiation incident on the earth's atmosphere, 1.34×10^{24} cal/yr. In this case, the average annual efficiency of net photosynthesis for all available solar radiation is $[(4.8 \times 10^{20} \text{ cal/yr})/(1.34 \times 10^{24} \text{ cal/yr})](100)$, or only 0.036%. This low figure averages in many biological deserts, e.g., open oceans, polar icecaps, winter landscapes, and arid regions. Also, not all solar radiation reaches the earth's surface, and much that does is in the infrared (cf. Fig. 4.2). Nevertheless, even with the apparently very low overall energy conversion factor, the trapping of solar energy by photosynthesis leads to the only source of free energy used to sustain life.

What is the highest possible efficiency for photosynthesis? For low levels of red light the conversion of radiant energy into the Gibbs free energy of photosynthetic products can be up to 34% in the laboratory (p. 216). Solar radiation incident on plants spans many wavelengths. Nearly half of the

radiant energy is in the region which can be absorbed by photosynthetic pigments, 400 to 700 nm (see p. 172 and Fig. 7.7). If all the incident wavelengths from 400 to 700 nm were absorbed by photosynthetic pigments, and 8 photons were required per CO_2 fixed, the maximum photosynthetic efficiency for the use of incident solar radiation would be just under half of 34%, say 15%. However, some sunlight is reflected or transmitted by plants (cf. Fig. 7.8), and some is absorbed by nonphotosynthetic pigments in the plant cells. Thus, the maximum photosynthetic efficiency for using incident solar energy under ideal conditions of temperature, water supply, and physiological status of plants in the field would be closer to 10%.

Actual measurements of photosynthetic efficiency in the field have indicated that up to about 7% of the incident solar energy can be stored in photosynthetic products for a rapidly growing crop under ideal conditions. Usually, the illumination level of the upper leaves of vegetation is too high for all excitations of the photosynthetic pigments to be used for the photochemistry of photosynthesis (see p. 241). The energy of many absorbed photons is therefore simply wasted as heat, especially when C-3 plants are illuminated with more than 10,000 lux (p. 334). Hence, the maximum sustained efficiency for the conversion of solar energy into Gibbs free energy stored in photosynthetic products is often near 3% for a crop when averaged over a day in the growing season (see Gates; see also Loomis *et al.*, cited in Ch. 8). For all vegetation averaged over a year, the efficiency is about 0.5%. This is consistent with our previous statement that about 1% of the radiant energy absorbed by photosynthetic pigments is stored in the products of photosynthesis, since only about half of the solar radiation incident on plants is absorbed by chlorophylls, carotenoids, and phycobilins.

Food chains and material cycles. We will now consider the fate of the Gibbs free energy stored in photosynthetic products when animals enter the picture. We begin by noting that across each step in a food chain the free energy decreases, as is required by the second law of thermodynamics. For instance, growing herbivores generally retain only 10 to 20% of the free energy of the ingested plant material, while a mature animal uses essentially all of its Gibbs free energy consumption just to remain in a state far from equilibrium. Growing carnivores will store about 10 to 20% of the free energy content of herbivores or other animals that they eat. Thus, there is a sizable loss in Gibbs free energy for each link in a food chain, and there are therefore seldom more than four steps in a food chain.

Although modern agriculture tends to reduce the length of man's food chain for meat, nevertheless, man puts a rather large demand on the Gibbs free energy available in the biosphere. The global average daily intake of

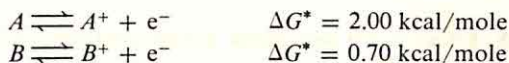
free energy is about 2400 kcal/person (see Gates). For a world population of 4 billion, the annual consumption of Gibbs free energy would be $(2.4 \times 10^6 \text{ cal/person-day})(365 \text{ days/yr})(4 \times 10^9 \text{ persons})$, or $4 \times 10^{18} \text{ cal/yr}$. Humans also consume plants and animals for clothing, shelter, firewood, papermaking, and in many other ways. However, man's food consumption alone amounts to almost 1% of the $4.8 \times 10^{20} \text{ cal}$ stored in photosynthetic products/yr. If man were to eat only carnivores which ate herbivores with a 10% retention in Gibbs free energy across each step in the food chain, he would indirectly be responsible for the consumption of the entire storage of energy by present day photosynthesis. Fortunately man obtains most of his free energy requirements from plants. The average daily consumption in the United States is 3100 kcal/person, of which 2200 kcal comes from plants and 900 kcal from animals. In turn, the animals consumed in this case require an average Gibbs free energy input of 6300 kcal of plant material, which corresponds to $(900/6300)(100)$, or 14% efficiency in free energy retention across this link in the food chain (see Cook).

The harnessing of solar radiation by photosynthesis starts the flow of Gibbs free energy through the biosphere. In addition to maintaining individual chemical reactions as well as entire plants and animals in a state far from equilibrium, the annual degradation of chemical energy to heat sets up various cycles (see Morowitz 1968). We have already indicated some of these, e.g., O_2 is evolved in photosynthesis and then consumed by respiration, while CO_2 cycles in the reverse direction between these two processes (Fig. 6.5). There is also a cycling between ATP and ADP + phosphate at the subcellular level, as well as the inevitable birth-death cycle of organisms. In addition, we can recognize a cycling of nitrogen, phosphorus, and sulfur in the biosphere. All of these material cycles can be regarded as a consequence of the unidirectional flow of Gibbs free energy, which becomes less after each step along the way.

Problems

6.1 A reaction $A + B \rightleftharpoons C$ has a ΔG^* of -4.09 kcal/mole of reactant or product at 25° . Assume that activity coefficients are unity. In which direction will the reaction proceed under the following conditions? (a) The concentrations of A , B , and C are all 1 molal. (b) The concentrations of A , B , and C are all 1 millimolal. (c) The concentrations of A , B , and C are all 1 micromolal. (d) What is the equilibrium constant for the reaction?

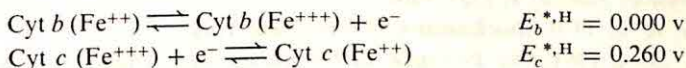
6.2 Consider the following two half-cell reactions at 25°:



Assume that the midpoint redox potential of the second reaction is +0.118 v and that all activity coefficients are unity. (a) If the redox potential of the B - B^+ couple is 0.000 v, what is the ratio of B^+ to B ? (b) What is the midpoint redox potential of the A - A^+ couple? (c) Suppose that all reactants and products are initially 1 molal but that the couples are in separate solutions of equal volume. If the half-cells are electrically connected with a metal wire, what is the initial electrical potential difference between them, and in which direction do electrons flow? (d) If all reactants and products are initially 1 molal, what is the concentration of each at equilibrium in a single solution? (e) Qualitatively, how would the answer to (d) change if the initial conditions were identical to (c), but as the electrons flow through the wire they do electrical work?

6.3 Suppose that in the dark 2 mM ADP and 5 mM phosphate occur inside chloroplasts, which are initially devoid of ATP. Assume that the temperature is 25° and that the pH is 7. (a) What is the ATP concentration at equilibrium? (b) When the chloroplasts are illuminated, the ADP concentration decreases to 1 mM. What is the new concentration of ATP, and what is the change in Gibbs free energy for continued photophosphorylation? (c) If ferredoxin has a redox potential of -0.580 v and the activity of NADPH_2 is 3% of that of NADP, what is the difference in redox potential between the two couples? (d) How much Gibbs free energy is available between the two couples in (c)? Is this enough for the continued formation of ATP under the conditions of (b)?

6.4 Consider the following two mitochondrial cytochromes:



Assume that the temperature is 25°, the chemical activity of Cyt b (Fe^{++}) is 20% of that of the oxidized form, and activity coefficients are equal to unity. (a) What is the redox potential of Cyt b ? (b) If the concentration of Cyt c (Fe^{++}) is 1 mM, what is the concentration of ferricytochrome c such that the Cyt c couple can just transfer electrons back to Cyt b ? (c) If the free energy to form 1 mole of ATP is 9.0 kcal in mitochondria, what must be the redox potential of Cyt c such that a coupling site using one electron per ATP could function between cytochromes b and c ? (d) If the 9.0 kcal gained upon the hydrolysis of one mole of ATP were used to transfer two moles of electrons in the energetically uphill direction from Cyt c to Cyt b , what would be the maximum redox potential of Cyt c ? (e) Assume that ATP is 0.2 mM, ADP is 0.5 mM, and phosphate is 4.0 mM in mitochondria. What is the answer to (d) using the Gibbs free energy from ATP hydrolysis under these conditions?

References

- Alberty, R. A. 1968. Effect of pH and metal ion concentration on the equilibrium hydrolysis of adenosine triphosphate to adenosine diphosphate. *Journal of Biological Chemistry* **243**: 1337-1343.
- Azzone, G. F., and S. Massari. 1971. Thermodynamic and kinetic aspects of the interconversion of chemical and osmotic energies in mitochondria. *European Journal of Biochemistry* **19**: 97-107.
- Castellan, G. W. 1971. *Physical Chemistry*, 2nd ed., Addison-Wesley, Reading (Mass.).
- Cook, E. 1971. The flow of energy in an industrial society. *Scientific American* **225**: No. 3 (September), 134-144. (Offprint No. 667.)
- Crofts, A. R., C. A. Wraight, and D. E. Fleischmann. 1971. Energy conservation in the photochemical reactions of photosynthesis and its relation to delayed fluorescence. *FEBS Letters* **15**: 89-100.
- Forrest, W. W., and D. J. Walker. 1971. The generation and utilization of energy during growth. *Advances in Microbial Physiology* **5**: 213-274.
- Forti, G., M. Avron, and A. Melandri (eds.). 1972. *Proceedings of the IIInd International Congress on Photosynthesis Research (Stressa)*, Junk, The Hague.
- Gates, D. M. 1971. The flow of energy in the biosphere. *Scientific American* **225**: No. 3 (September), 88-100. (Offprint No. 664.)
- Hayaishi, O. (ed.). 1973. *Molecular Mechanisms of Oxygen Activation*, Academic Press, New York.
- Ikuma, H. 1972. Electron transport in plant respiration. *Annual Review of Plant Physiology* **23**: 419-436.
- Knox, R. S. 1969. Thermodynamics and the primary processes of photosynthesis. *Biophysical Journal* **9**: 1351-1362.
- Lehninger, A. L. 1970. *Biochemistry*, Worth, New York.
- MacInnes, D. A. 1961. *The Principles of Electrochemistry*, Dover, New York.
- Morowitz, H. J. 1968. *Energy Flow in Biology*, Academic Press, New York.
- Morowitz, H. J. 1970. *Entropy for Biologists—An Introduction to Thermodynamics*, Academic Press, New York.
- Morris, J. G. 1968. *A Biologist's Physical Chemistry*, Addison-Wesley, Reading (Mass.).
- Nobel, P. S. 1973. Free energy: The currency of life, in *Nature in the Round*, N. Calder, ed., Weidenfeld & Nicolson, London, pp. 194-207.
- Palmer, J. M., and D. O. Hall. 1972. The mitochondrial membrane system. *Progress in Biophysics and Molecular Biology* **24**: 125-176.
- Payne, W. J. 1970. Energy yields and growth of heterotrophs. *Annual Review of Microbiology* **24**: 17-52.
- Penning de Vries, F. W. T. 1972. Respiration and growth, in *Crop Processes in Controlled Environments*, A. R. Rees, K. E. Cockshull, D. W. Hand, and R. G. Hurd, eds., Academic Press, London, pp. 327-347.

- Ross, R. T., and M. Calvin. 1967. Thermodynamics of light emission and free-energy storage in photosynthesis. *Biophysical Journal* 7: 595-614.
- Ryther, J. H. 1969. Photosynthesis and fish production in the sea. *Science* 166: 72-76.
- San Pietro, A. (ed.). 1972. *Photosynthesis and Nitrogen Fixation* (Vol. XXIV, Part B, in the series *Methods in Enzymology*), Academic Press, New York.
- Sanadi, D. R. (ed.). 1971. *Current Topics in Bioenergetics*, Vols. 3 and 4, Academic Press, New York.
- Schwartz, M. 1971. The relation of ion transport to phosphorylation. *Annual Review of Plant Physiology* 22: 469-484.
- Spanner, D. C. 1964. *Introduction to Thermodynamics*, Academic Press, New York.
- Tager, J. M., S. Papa, E. Quagliariello, and E. C. Slater (eds.). 1970. *Electron Transport and Energy Conservation*, Adriatica Editrice, Bari.
- van Dam, K., and A. J. Meyer. 1971. Oxidation and energy conservation by mitochondria. *Annual Review of Biochemistry* 40: 115-160.
- Wainio, W. W. 1970. *The Mammalian Mitochondrial Respiratory Chain*, Academic Press, New York.
- Weyer, E. M. (ed.). 1968. Bioelectrodes. *Annals of the New York Academy of Sciences* 148: Art. 1, 1-287.
- White, A., P. Handler, and E. L. Smith. 1973. *Principles of Biochemistry*, 5th ed., McGraw-Hill, New York.
- Witt, H. T. 1971. Coupling of quanta, electrons, fields, ions and phosphorylation in the functional membrane of photosynthesis. *Quarterly Reviews of Biophysics* 4: 365-477.
- Woodwell, G. M. 1970. The energy cycle of the biosphere. *Scientific American* 223: No. 3 (September), 64-74. (Offprint 1190.)
- Zimmerman, A. M. (ed.). 1970. *High Pressure Effects on Cellular Processes*, Academic Press, New York.

LEAVES

In this chapter we will draw upon a number of previously introduced topics in order to discuss gas and energy fluxes for leaves. For example, we will reconsider the transpiration of water and the photosynthetic fixation of carbon dioxide. We will quantitatively discuss the movement of such gases into and out of leaves in terms of the various resistances involved. The use of resistances to quantitatively describe gas fluxes was first applied to leaves by H. T. Brown and F. Escombe in 1900. Over the years, consideration of resistance networks has become very convenient for specifying which parts of the pathway are most limiting for photosynthesis or transpiration. The driving forces for the various fluxes are the differences in carbon dioxide or water vapor concentrations and the differences in temperature. Although we will usually be referring to leaves, the discussion of gas and energy fluxes is also applicable to petals, sepals, stems, and other plant parts.

We should emphasize at the outset that individual plants as well as environmental conditions vary tremendously. Thus, in this and the next chapter we will be indicating an approach to the study of plant physiology and physiological ecology, rather than a compendium of facts suitable for all situations. However, certain basic features should become obvious. For instance, the carbon dioxide uptake during photosynthesis is accompanied by a water efflux through the stomates. Such water loss during transpiration serves to cool the leaf. However, a leaf balances its energy influxes against effluxes mainly by changing its temperature, which affects the amount of radiation emitted by the leaf as well as the heat conducted to the surrounding air. Another generality is that leaf temperatures for small leaves tend to be closer to those of the air than do the temperatures of large leaves. Since it is difficult to appreciate the relative contributions of the various factors without first making calculations, we will use representative values for leaf

and environmental parameters in order to describe quantitatively the gas fluxes and the energy balance of leaves. Hopefully, we can then begin to understand the consequences of certain adaptations of plants to their environment as well as recognize the experimental data needed for future refinements of our calculations.

Resistances

The resistances discussed in this section are those encountered by water vapor as it diffuses from the pores of the cell walls of mesophyll cells out into the turbulent air surrounding a leaf. We will define resistances for the intercellular air spaces, the stomates, the cuticle, and the unstirred boundary layer next to a leaf.* As we will explain later in this chapter, CO_2 diffuses across the same gaseous phase resistances as water vapor, and also across a number of others in the liquid phases of mesophyll cells.

Throughout this book we have used equations of the following general form: flux = proportionality coefficient $\times$ force (e.g., pp. 11, 102). The proportionality coefficients in such flux expressions are measures of conductivity. We might equally well choose an alternative form for the relation between forces and fluxes: flux = force/resistivity, where resistivity is simply the reciprocal of the conductivity. In this chapter and the next we are more concerned with resistance, a quantity proportional to the resistivity.

What is the difference between resistivity and resistance? Resistivity usually refers to a fundamental physical property of a material, e.g., electrical resistivities are tabulated in suitable handbooks. On the other hand, electrical *resistance* describes a particular component in an electrical circuit, and we can conveniently measure its magnitude in the laboratory. Electrical resistivity (ρ) generally has the units of ohm-cm, while electrical resistance ($R = \rho\Delta x/A$, cf. p. 102) is expressed in ohms. Thus, resistance and resistivity differ in their units. In fact, much of our attention in this chapter will be devoted to the units for parameters such as resistance, since many different systems are in use in plant physiology, ecology, meteorology and other related disciplines. Some conversion factors of use in this chapter are given in Table 7.2.

Resistance of unstirred boundary layer adjacent to leaf. As a starting point for our discussion of resistances of air layers, let us consider the

* Frictional interactions between a fluid such as air and a solid such as a leaf lead to an unstirred layer in the fluid adjacent to the solid phase (p. 27).

one-dimensional form of Fick's first law of diffusion ($J_j = -D_j \partial c_j / \partial x$, Eq. 1.1). As in Chapter 1, we will replace the concentration gradient by the difference in concentration across some distance. In effect, this means that we are going to consider cases not too far from equilibrium, so that the flux will depend linearly on the force and the force can be represented by the difference in concentration. The distance which interests us here is the one occurring across the unstirred air layer adjacent to the surface of a leaf, δ^a . Consequently, Fick's first law assumes the following form for the diffusion of species j :

$$\begin{aligned} J_j &= -D_j \frac{\partial c_j}{\partial x} \cong D_j \frac{\Delta c_j}{\Delta x} \\ &= D_j \frac{\Delta c_j^a}{\delta^a} \\ &= \frac{\Delta c_j^a}{R_j^a} \end{aligned} \quad (7.1)$$

Equation 7.1 is a very general relation showing how the net flux of species j depends on its diffusion coefficient, D_j , and on the difference in its concentration (Δc_j^a) across a distance δ^a of the air. The net flux (J_j) is of course toward regions of lower c_j , as we will specifically consider for water vapor and CO_2 . Outside the unstirred air layer of thickness δ^a is a region of air turbulence, where we will assume that the concentrations are the same as in the bulk atmosphere (an assumption that we will remove in the next chapter). Equation 7.1 indicates that J_j equals Δc_j^a divided by a resistance, R_j^a , an aspect we will consider next.

The unstirred air layers on both sides of a leaf represent important barriers in the pathways for the entry of CO_2 and the exit of H_2O , as was clearly shown by K. Raschke in the 1950s. Movement across such layers is by diffusion of the gas molecules in response to differences in concentration. From Equation 7.1, we can represent the resistance of a boundary layer of air, R_j^a , as follows:

$$R_j^a = \frac{\Delta c_j^a}{J_j} = \frac{\delta^a}{D_j} \quad (7.2)$$

Since the customary units for the diffusion coefficient (D_j) are cm^2/sec , and the thickness of the unstirred layer δ^a is suitably expressed in cm , R_j^a is usually given in $\text{cm}/(\text{cm}^2/\text{sec})$, or sec/cm . Other systems of units are sometimes used for R_j^a , e.g., min/cm ($1 \text{ min}/\text{cm} = 60 \text{ sec}/\text{cm}$). We should point out that D_j is a fundamental measure of conductivity (values available in suitable handbooks), which describes the diffusion of species j in some

medium or material. On the other hand, δ^a characterizes a particular situation, since the thickness of the unstirred layer depends on wind velocity and leaf size, as we will discuss below. Thus, R_j^a as defined by Equation 7.2 describes a particular component of the path, analogous to the resistance (R) used in Ohm's law. Since J_j is expressed per unit leaf area, R_j^a also relates to unit area of the leaf.

Recalling Equation 1.9 ($P_j = D_j K_j / \Delta x$), we recognize that D_j / δ^a is the permeability coefficient for species j , P_j , as it diffuses across an unstirred air layer of thickness δ^a . Thus, R_j^a as defined by Equation 7.2 is equal to $1/P_j$, indicating that we have been dealing with the reciprocals of resistances whenever we were discussing permeability coefficients. When something readily crosses a region of air by diffusion, P_j is large and R_j^a is small; when the average rate of diffusion is low, R_j^a will be correspondingly large. Using resistances, we can describe gas fluxes into and out of leaves employing a number of relations which were originally developed for the analysis of electrical circuits (see Gaastra; Monteith in Evans, and Lake).

Unstirred layer thickness. The quantity δ^a represents the *average* thickness of the unstirred air layer adjacent to a leaf. The boundary layer is generally somewhat thinner at the edges of a leaf than at its center. The main factors affecting δ^a are the ambient wind velocity and the leaf size, with leaf shape exerting a secondary influence. Partly for convenience, but mainly since it has proved experimentally justifiable, we will handle the effect of the leaf size on δ^a by l^{leaf} , the maximum dimension of the leaf in the direction of the wind. Based on hydrodynamic theory for laminar flow adjacent to a flat surface as modified by actual observations on leaves, there is then an approximate, but convenient expression for the average thickness of the unstirred layer next to a leaf:

$$\delta_{(\text{cm})}^a \cong 0.4 \sqrt{\frac{l_{(\text{cm})}^{\text{leaf}}}{v_{(\text{cm/sec})}^{\text{wind}}}} \quad (7.3)$$

where $l_{(\text{cm})}^{\text{leaf}}$ is the linear dimension of the leaf in cm in the downwind direction, $v_{(\text{cm/sec})}^{\text{wind}}$ is the ambient wind velocity in cm/sec, and $\delta_{(\text{cm})}^a$ is the average thickness in cm of the unstirred layer adjacent to the leaf. The factor 0.4 has dimensions of $\text{cm sec}^{-1/2}$, which we can deduce by using the indicated units for the three variables.

Instead of the numerical factor 0.4 in Equation 7.3, hydrodynamic theory actually predicts a factor closer to 0.6 for a finite plane.* However, wind

* For a *semi-infinite* plane, the factor is 1.2 to 1.3 (see Gates and Papian; Linacre; and Raschke).

tunnel measurements and other experiments have indicated that 0.4 is actually a more suitable factor for leaves. This divergence from theory relates to the relatively small size of leaves, their irregular shape, leaf curl, and phenomena such as leaf flutter. Moreover, the dependency of δ^a on $(l^{\text{leaf}})^{0.5}$, which applies to large flat surfaces, does not always give the best fit to the experimental data; wind tunnel measurements for various leaf shapes and sizes give values of the exponent from 0.3 to 0.5. Various shape considerations, including the leaf dimension perpendicular to the wind direction, can modify these relations even further. In addition, instead of the dependence on the inverse square root of wind velocity indicated in Equation 7.3, actual measurements with leaves are best described by exponents from -0.5 to -0.7 . Consequently, Equation 7.3 must be regarded as only a useful approximation for indicating how the average boundary layer thickness varies with leaf size and wind velocity. It turns out that Equation 7.3 can also be used to give approximate values of δ^a for conifer needles if the diameter of the needle is used for l^{leaf} .

Because the ambient wind speed helps control the thickness of the unstirred air layer adjacent to a leaf, it influences R_j^a ($R_j^a = \delta^a/D_j$, Eq. 7.2). Thus, we should have some idea of the magnitudes of wind velocities that might be expected in nature. The lowest wind velocities encountered, which represent so-called "still air," are about 10 cm/sec (1 km/hr = 27.8 cm/sec and 1 mile/hr = 44.7 cm/sec, App. III). The highest wind velocity commonly experienced by plants is about 10 m/sec (approximately 36 km/hr or 22 miles/hr), while the more usual wind speeds across exposed plant leaves are 1 to 2 m/sec. The most common units for wind velocities and their conversion factors are given in Table 7.2. Since the thickness of the unstirred air layer enters into many calculations for heat movement and gas fluxes into and out of leaves, the magnitudes of $\delta^a_{(\text{cm})}$ for a wide variety of wind speeds and leaf dimensions are presented in Table 7.1.

To conclude this section, we will estimate values that might be expected for R_{wv}^a , the resistance for water vapor diffusing across the unstirred air layer adjacent to a leaf. Let us take a thick unstirred air layer of 0.25 cm and a relatively thin one of 0.025 cm (cf. values in Table 7.1). For water vapor diffusing in air at 20°, D_{wv} is 0.25 cm²/sec (App. II). Using Equation 7.2 ($R_j^a = \delta^a/D_j$), we calculate that R_{wv}^a is (0.25 cm)/(0.25 cm²/sec), or 1.0 sec/cm for the thick unstirred layer and 0.1 sec/cm for a δ^a of 0.025 cm. These values for boundary layer resistance are usually less than those for stomatal resistance, which we will now examine.

Stomata. As we indicated in Chapter 1 (p. 4), the stomata help control the exit of water vapor from leaves and the entry of CO₂ into them. Although the epidermal cells occupy a much greater fraction of the leaf surface area

Table 7.1

Thickness in cm of the unstirred air layer adjacent to a leaf, $\delta^a_{(\text{cm})}$, for a variety of leaf sizes and wind velocities. Calculations were made assuming that $\delta^a_{(\text{cm})} = 0.4 \sqrt{l^{\text{leaf}}_{(\text{cm})}/v^{\text{wind}}_{(\text{cm/sec})}}$ (Eq. 7.3), where $l^{\text{leaf}}_{(\text{cm})}$ is the linear dimension of the leaf in the wind direction in cm, and $v^{\text{wind}}_{(\text{cm/sec})}$ is the ambient wind velocity in cm/sec. (Note that 1 km/hr = 27.8 cm/sec and 1 mile/hr = 44.7 cm/sec, App. III.)

		$v^{\text{wind}}_{(\text{cm/sec})}$						
		10	28	45	100	278	447	1000
$l^{\text{leaf}}_{(\text{cm})}$	0.2	0.057	0.034	0.027	0.0179	0.0107	0.0085	0.0057
	1	0.127	0.076	0.060	0.040	0.024	0.0189	0.0127
	5	0.28	0.169	0.133	0.089	0.054	0.042	0.028
	25	0.63	0.38	0.30	0.20	0.120	0.095	0.063
	50	0.89	0.54	0.42	0.28	0.170	0.134	0.089

than do the stomatal pores, the waxy cuticle covering them greatly reduces the water loss from their cell walls to the turbulent air surrounding the leaf, the so-called *cuticular* transpiration. The usual pathway for water vapor leaving a leaf during transpiration is therefore through the stomata.

The stomatal aperture is controlled by the conformation of the two guard cells surrounding a pore (cf. Figs. 1.2 and 7.1). These cells are kidney-shaped, may be 40 μm long, and, unlike ordinary epidermal cells, they contain chloroplasts. When the guard cells are relatively flaccid the stomatal pore is nearly closed, as is the case for most plants at night. The actual mechanism for the response of guard cells to light is not yet fully understood. However, modern work indicates that upon illumination guard cells take up K^+ , which may increase by 0.1 to 0.3 M in a cell (see Fischer); K^+ may be actively transported into the cells using ATP produced in the light. To maintain electroneutrality, chloride or organic ions may accompany the K^+ uptake into the guard cells. The uptake of ions can raise the internal osmotic pressure by 3 to 10 bars, and thus lower the internal water potentials; water then spontaneously flows from the epidermal cells into the guard cells. Such water entry leads to an increase in the internal hydrostatic pressure of a pair of guard cells, which in turn causes them to expand and their opposed cell walls (those on either side of the pore) to become concave. As the kidney-shaped guard cells thus bow outward, an elliptical pore develops between the two cells. The formation of this pore is a consequence of the anisotropic properties of the cell wall surrounding each guard cell (see Aylor *et al.*). The distance between the guard cells across the open pore is generally 5 to 10 μm , and the major axis of the elliptical pore may be about 20 μm .

For dicots, stomates are usually more numerous on the lower surface than on the upper surface of the leaves. In some such species, stomata may even be nearly absent from the upper epidermis. On the other hand, many grasses have approximately the same number of stomates per unit area on each side. A frequency of 4,000 to 30,000 stomates per cm^2 is representative for the lower surface of most leaves. The pores of the open stomata generally occupy 0.4 to 2% of the leaf surface area. Thus, the area for diffusion of gases through the stomatal pores in the upper or lower epidermis of a leaf (A^{st}) is much less than the leaf surface area, A (see Meidner and Mansfield).

Stomatal resistance. We can apply Fick's first law in the form of Equation 7.1 ($J_j = D_j \Delta c_j / \Delta x$) to describe the diffusion of gases along stomatal pores. We will let the depth of the stomatal pore be d^{st} (see Fig. 7.1); the concentration of species j changes by Δc_j^{st} along the distance d^{st} . In the steady state, the rate of movement of species j toward or away from the stomates (J_j times the leaf area A) must be equal to the rate of movement of species j through the stomates (the flux within the stomates, $D_j \Delta c_j^{\text{st}} / d^{\text{st}}$, times the stomatal area A^{st} which occurs for leaf area A). This leads us to the obvious but extremely important conclusion that the stomates constrict the area available for the diffusion of the gas molecules. Recognition of this constricting effect of the stomates on the flux of species j then gives us the following relations:

$$\begin{aligned} J_j &= D_j \frac{\Delta c_j^{\text{st}}}{d^{\text{st}}} \frac{A^{\text{st}}}{A} \\ &= \frac{D_j n a^{\text{st}}}{d^{\text{st}}} \Delta c_j^{\text{st}} \end{aligned} \quad (7.4)$$

where n is the number of stomates per unit area of the leaf and a^{st} is the average area per stomatal pore, i.e., na^{st} equals the fraction of the leaf surface area occupied by stomatal pores, A^{st}/A . The flux J_j in Equation 7.4 refers to the rate of movement of species j *per unit area of the leaf*, a quantity which is considerably easier to measure than the flux within a stomatal pore.

The area available for water vapor diffusion abruptly changes from A^{st} to A at the leaf surface. On the other hand, the three-dimensional surfaces of equal concentration must fan out from each stomatal pore (Fig. 7.1). This geometrical aspect could introduce considerable complications, but fortunately we can still use a one-dimensional form for Fick's first law to describe gases moving across the unstirred layer next to a leaf. The distance between stomates is often about $100 \mu\text{m}$, which is considerably less than the thicknesses of nearly all unstirred layers (cf. Table 7.1). Thus, the three-

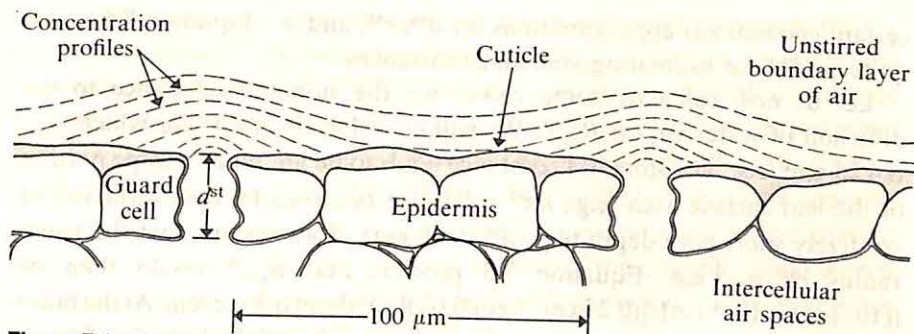


Figure 7.1

Concentration profiles of water vapor outside open stomata. A stomatal pore has a mean depth (d^{st}) and an area (a^{st}) equal to $\pi(r^{\text{st}})^2$, where r^{st} is the mean "radius" of the pore.

dimensional concentration contours from adjacent stomata tend to overlap in the unstirred layer (Fig. 7.1). When this occurs, the concentration of water vapor varies only slightly in the y - and the z -directions, but continuously drops off in the x -direction (the direction perpendicular to the leaf surface), and we can therefore generally use a one-dimensional form of Fick's first law (see Meidner and Mansfield). However, the concentration patterns on both ends of the stomatal pore cause the pore to have an effective depth greater than d^{st} by about the mean, or effective, "radius" of the pore, r^{st} . Since stomatal pores are approximately elliptical, not circular, we will define r^{st} as follows: $\pi(r^{\text{st}})^2 = a^{\text{st}}$, where a^{st} is the effective area of the pore.

For our subsequent applications to transpiration and photosynthesis, we will find it convenient to define a stomatal resistance for the diffusion of species j , R_j^{st} . We have previously described the resistance of the unstirred air layer R_j^{a} based on a form of Fick's first law for the diffusion of species j ($R_j^{\text{a}} = \Delta c_j^{\text{a}}/J_j = \delta^{\text{a}}/D_j$, Eq. 7.2). In an analogous fashion, we will use Equation 7.4 to define the resistance for the diffusion of gases along the length of the stomates:

$$R_j^{\text{st}} = \frac{\Delta c_j^{\text{st}}}{J_j} = \frac{d^{\text{st}} + r^{\text{st}}}{D_j n a^{\text{st}}} \quad (7.5)$$

Equation 7.5 also incorporates the effective depth of the stomatal pore, $d^{\text{st}} + r^{\text{st}}$, where r^{st} is the mean pore radius just introduced. When the width of a stomatal pore is a few tenths of a micron, as occurs when the pores are nearly closed, the mean free path for molecules diffusing in air is about the same as the dimensions of the opening. In this case, the molecular interaction with the sides of the stomatal pore is important, and this affects the value of D_j . Even though we generally ignore this interaction and make

certain geometrical approximations for d^{st} , r^{st} , and a^{st} , Equation 7.5 is still quite useful for estimating stomatal resistances.

Let us now calculate some values for the stomatal resistance to the diffusion of water vapor, $R_{\text{wv}}^{\text{st}}$. We will consider air at 20° for which D_{wv} is $0.25 \text{ cm}^2/\text{sec}$. The stomatal resistance tends to be low when a large portion of the leaf surface area (e.g., $na^{\text{st}} = 0.02$) is occupied by open stomates of relatively short pore depth (e.g., $d^{\text{st}} = 10 \mu\text{m}$). If we assume that the mean radius r^{st} is $5 \mu\text{m}$, Equation 7.5 predicts that $R_{\text{wv}}^{\text{st}}$ would then be $[(10 + 5) \times 10^{-4} \text{ cm}] / [(0.25 \text{ cm}^2/\text{sec})(0.02)]$, or about 0.3 sec/cm . At the other extreme, the open stomates may occupy only 0.4% of the lower surface of a leaf ($na^{\text{st}} = 0.004$), and the pore depth may be rather large, e.g., $50 \mu\text{m}$. Again assuming that r^{st} is $5 \mu\text{m}$, the stomatal resistance calculated using Equation 7.5 is $[(50 + 5) \times 10^{-4}] / [(0.25)(0.004)]$, or 5.5 sec/cm , a rather large value for open stomates.

The stomatal resistance usually has values from 0.5 to 5 sec/cm for water vapor diffusing out through the open stomates of most mesophytes. In general, agricultural crops tend to have relatively low values for $R_{\text{wv}}^{\text{st}}$, while for certain xerophytes with open stomates it can go up to about 20 sec/cm . Moreover, some xerophytes adapt to arid environments by having sunken stomates, which leads to another resistance in series with $R_{\text{wv}}^{\text{st}}$ and the overall resistance for water loss in transpiration is then increased. Of course, as the stomatal pores close the resistance increases correspondingly, since R_j^{st} is inversely proportional to the stomatal area by Equation 7.5. Since R_j^{st} is essentially the only resistance in the whole diffusion pathway which is variable over a wide range, changes of the openings of the stomatal pores provide a plant with an important control mechanism for adjusting the movement of gases into or out of the leaves.

Cuticular resistance. Some water molecules diffuse across the waxy cuticle of the epidermal cells, the so-called cuticular transpiration. This movement of H_2O across the cuticle probably involves liquid water as well as water vapor. We will identify a resistance for the cuticle, R_j^{c} , the cuticular resistance for the diffusion of species j . This R_j^{c} is in parallel with R_j^{st} , the stomatal resistance. The resistance for cuticular transpiration (R_{wv}^{c}) generally ranges from 30 sec/cm to 200 sec/cm for different plants. Hence, the cuticular resistance is usually much larger than that of open stomates. When the stomates are nearly closed (high $R_{\text{wv}}^{\text{st}}$), the cuticular transpiration can exceed the loss of water through the stomates. Also, if the cuticle is mechanically damaged or develops cracks, as can occur for older leaves, R_j^{c} can be lowered and more water will then move out of the leaf by this pathway.

Resistance of intercellular air spaces. Another resistance encountered

in plant leaves is R_j^{ias} , the resistance of the intercellular air spaces for the diffusion of species j . Because of the irregular shape of such air spaces, which generally account for about 50% of the leaf volume, it is difficult to estimate R_j^{ias} accurately from geometrical considerations alone. However, the intercellular air spaces do act like an unstirred air layer, hence we will describe R_j^{ias} by a relation similar to Equation 7.2 ($R_j^a = \delta^a/D_j$). As a first approximation, we can regard the thickness of the unstirred layer in the present case as an "average" distance from the surfaces of the mesophyll cells within a leaf to the inner side of a stomatal pore, δ^{ias} . By analogy with Equations 7.2 and 7.4, we can then express R_j^{ias} as

$$R_j^{ias} = \delta^{ias}/D_j \quad (7.6)$$

For convenience, we will combine a number of factors to get an effective distance, δ^{ias} . For instance, we will let δ^{ias} include the effective length of the cell wall pores where the evaporation of water or the dissolving of CO_2 takes place. This length is greater than the actual distance along the cell wall interstices, since we must correct for the diminution of the cross-sectional area available for diffusion, a diminution caused by the presence of the nongaseous parts of the cell wall. Also, δ^{ias} includes the effective length of the thin waxy layer which generally occurs on the surfaces of mesophyll cells. In addition, δ^{ias} incorporates the fact that the entire cross-section of the mesophyll region is not available for diffusion of gases, but that the flow is constricted to the intercellular air spaces, i.e., $A^{ias}/A < 1$. Thus, that part of the δ^{ias} which refers to the intercellular air spaces *per se* equals the actual distance involved times the ratio A/A^{ias} . If the mesophyll region were about half air by volume (a rather typical value for a leaf), A/A^{ias} would be approximately two.

The effective length δ^{ias} , including all of the factors just enumerated, might range from 100 μm to 1 mm for most leaves. Equation 7.6 indicates therefore that R_{wv}^{ias} might be from $(100 \times 10^{-4} \text{ cm})/(0.25 \text{ cm}^2/\text{sec})$, or 0.04 sec/cm, to $(0.1)/(0.25)$, or 0.4 sec/cm ($D_{wv} = 0.25 \text{ cm}^2/\text{sec}$ at 20° , App. II). The main point which we want to emphasize here is that the resistance of the intercellular air spaces generally is relatively small compared with the other resistances encountered by gases diffusing into or out of leaves.

Fick's first law and resistances. The form of Fick's first law which we are using to describe the flux of water vapor out of a leaf or carbon dioxide into one is appropriate for a one-dimensional situation (e.g., $J_j = D_j \Delta c_j / \Delta x$, Eq. 7.1). The water vapor lost from a leaf during transpiration emanates from the cell walls of its mesophyll cells. We can imagine that this water vapor moves in the intercellular air spaces from the center plane of the leaf

toward the leaf surface by diffusing down planar fronts of successively lower concentration (see Figs. 7.1 and 7.3). Our imaginary planar fronts are parallel to the leaf surface, so that the direction for the fluxes is perpendicular to the leaf surface. When we reach the inner side of a stomatal pore, the area for diffusion is reduced from A to A^{st} . In other words, we are still discussing the advance of planar fronts in one dimension, but the flux is now constricted to the stomatal pores. (A small amount of water comprising the cuticular transpiration diffuses across the cuticle in parallel with the stomatal fluxes.) Finally, the movement of water vapor across the unstirred layer at the leaf surface is again a one-dimensional diffusion process, in this case across a distance δ^a . Thus, all fluxes of gases which we will consider here are moving perpendicular to the leaf surface (in contrast with those in the leaf xylem or phloem which occur axially within the leaf). We will find it convenient to express all gas fluxes on the basis of unit leaf area. All resistances are also given per unit leaf area, as we mentioned above.

In the next two sections we will consider the various resistances involved in transpiration and photosynthesis, but we should first determine whether resistance defined by flux equations of the form $J_j = \Delta c_j / R_j$ are really suitable for discussing the fluxes of gases. Specifically, is R_j essentially independent of c_j ? If this resistance should vary significantly with concentration, then we should find another way to express the force. We could, for example, employ $J_j = \Delta \mu_j / R'_j$ as our flux equation, where $\Delta \mu_j$ is the difference in chemical potential of species j (of course, R'_j would have different units than R_j). The crux of the matter is whether R_j depends on c_j , since a concentration difference is a very convenient quantity by which to represent the force on gaseous species j . If R_j does depend on c_j , the functional form of the flux equation, $J_j = \Delta c_j / R_j$, is still correct, but R_j would change as c_j changes, and the relation would not be very useful. The argument that R_j is independent of c_j for ideal gases is somewhat involved, but its importance warrants a brief treatment here.

We will begin by writing the equation for the chemical potential of species j in a gas phase, μ_j^{vapor} . By Equations 2.21 and App. VII.10, μ_j^{vapor} equals $\mu_j^* + RT \ln (P_j / P_j^*)$, where P_j is the actual partial pressure of species j in the gas phase, while P_j^* is its maximum possible partial pressure at that temperature and one atmosphere of total pressure. (We are ignoring the gravitational term of μ_j^{vapor} , since it does not influence the fluxes of gases into or out of leaves.) At constant T the force promoting the movement of species j in the x -direction, $-\partial \mu_j^{\text{vapor}} / \partial x$, is $-RT \partial [\ln (P_j / P_j^*)] / \partial x$, which equals $-(RT / P_j) \partial P_j / \partial x$. For an ideal gas, $P_j V$ equals $n_j RT$. The total number of moles of species j divided by the volume (n_j / V) is the concen-

tration of species j , c_j , hence $P_j (= n_j RT/V)$ can be replaced by $c_j RT$. Making this substitution for P_j and using Equation 3.6, $J_j = u_j c_j (-\partial \mu_j / \partial x)$, we can express the flux of gaseous species j as follows:

$$\begin{aligned} J_j &= u_j c_j \left(\frac{-\partial \mu_j^{\text{vapor}}}{\partial x} \right) = -u_j c_j \frac{RT}{P_j} \frac{\partial P_j}{\partial x} \\ &= -u_j c_j \frac{1}{c_j} RT \frac{\partial c_j}{\partial x} = -u_j RT \frac{\partial c_j}{\partial x} \\ &\cong \frac{u_j RT}{\Delta x} \Delta c_j \end{aligned} \quad (7.7)$$

where in the last line of the equation we have replaced the negative concentration gradient, $-\partial c_j / \partial x$, by a suitably defined average concentration gradient, $\Delta c_j / \Delta x$.

In essence, Equation 7.7 represents a thermodynamic derivation of Fick's first law for a gas phase, since it has the same form as Equation 7.1 ($J_j = D_j \Delta c_j / \Delta x$). More importantly, Equation 7.7 is of the form, $J_j = \Delta c_j / R_j$, where R_j is $\Delta x / (u_j RT)$. Thus, we can describe the diffusion of species j in a gas phase in terms of a resistance R_j which is independent of concentration (the slight dependence of u_j on concentration can be ignored as a first approximation). On the other hand, if we replace $-\partial \mu_j^{\text{vapor}} / \partial x$ by $\Delta \mu_j^{\text{vapor}} / \Delta x$, Equation 7.7 then becomes J_j equals $(u_j c_j / \Delta x) \Delta \mu_j^{\text{vapor}}$ or $\Delta \mu_j^{\text{vapor}} / R_j'$, where R_j' is $\Delta x / (u_j c_j)$. The resistance R_j' thus depends inversely on the concentration, an extremely inconvenient situation for quantitatively discussing the fluxes of gases into and out of leaves. Hence, resistances appropriate for the flux equation $J_j = \Delta c_j / R_j$ rather than the expression $J_j = \Delta \mu_j / R_j'$ have come into widespread use in descriptions of transpiration and photosynthesis, since the former resistances are essentially independent of concentration.

Water vapor fluxes accompanying transpiration

Here we will describe the flux of water vapor out of a leaf during transpiration in terms of the resistances involved. We will incorporate the resistances into a resistance network, then indicate the general values for the various components and the differences in water vapor concentration across them. Our analysis of water vapor fluxes will not only indicate the degree of control of transpiration exercised by the stomates compared with the effect of the unstirred air layer adjacent to a leaf, but it will also be useful when we

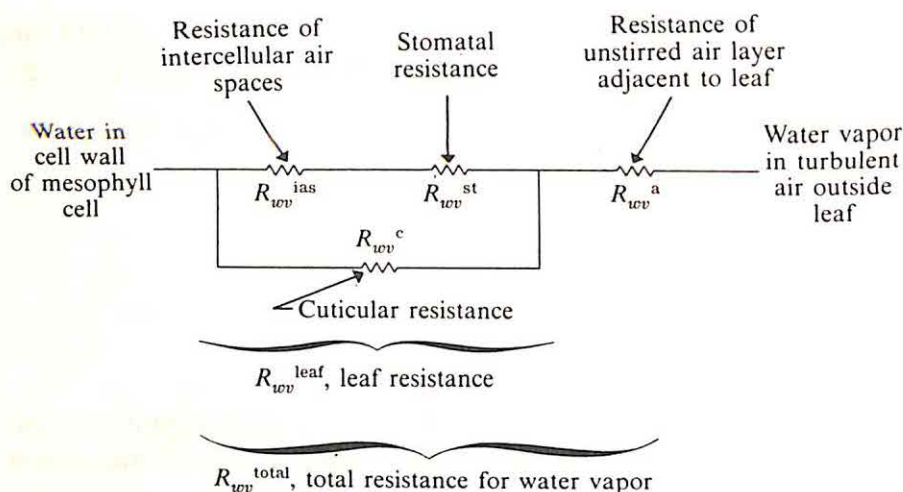


Figure 7.2

Resistances involved in water vapor flow accompanying transpiration, as arranged in an electrical circuit analog.

later discuss the heat flux accompanying the evaporation of water during transpiration.

Resistance network. Water vapor which evaporates from the cell walls of leaf mesophyll cells diffuses through the intercellular air spaces to the stomates and then into the outside air. We have already introduced the resistances encountered in this pathway, viz., R_{wv}^{ias} , R_{wv}^{st} , R_{wv}^c , and R_{wv}^a . Figure 7.2 illustrates how these four resistances are arranged using an electrical circuit analog.

The resistance R_{wv}^{ias} includes all parts of the pathway from the site of water evaporation to the leaf epidermis. The water evaporating along the interstices of the cell walls of mesophyll cells first diffuses out of these tortuous pores. Next, the water vapor moves across a thin waxy layer generally found on the cell walls of the mesophyll cells; this layer is often as much as $0.1 \mu\text{m}$ thick (see Esau). After crossing the waxy layer the water vapor diffuses through the intercellular air spaces and then through the stomates (resistance = R_{wv}^{st}) to reach the underside of the unstirred layer adjacent to the leaf surface. Alternatively, water in the cell walls of mesophyll cells might move as a liquid to the cell walls on the cuticle side of epidermal cells, where it could evaporate and the vapor then diffuse across the cuticle (or water may move across the cuticle as a liquid) before reaching the unstirred layer adjacent to the leaf. The pathway for such cuticular transpiration (resistance = R_{wv}^c) is in parallel with the transpiration which occurs

through the stomatal pores. For simplicity, we have not included in Figure 7.2 the intermediate case of water evaporating from the cell walls of mesophyll cells and then moving through the intercellular air spaces before crossing the waxy cuticle (the resistance of this pathway is indistinguishable from R_{wv}^c , since $R_{wv}^c \gg R_{wv}^{ias}$). The final resistance encountered by the diffusing water vapor is that of the unstirred air layer just outside the leaf, R_{wv}^a .

As we can see from Figure 7.2, the resistances R_{wv}^{ias} and R_{wv}^{st} occur in series. The total resistance R of a group of resistors in series is simply the sum of the resistances ($R = \sum_i R_i$, where R_i is the resistance of series

resistor i). Thus, the resistance of the pathway from the mesophyll cell walls across the intercellular air spaces and through the stomates is $R_{wv}^{ias} + R_{wv}^{st}$. This resistance is in parallel with R_{wv}^c , the cuticular resistance. The reciprocal of the total resistance of a group of resistors in parallel is the sum of the reciprocals of the individual resistances ($1/R = \sum_i 1/R_i$, where R_i is

the resistance of parallel resistor i). For two resistors in parallel, R is equal to $R_1 R_2 / (R_1 + R_2)$. Since $(R_{wv}^{ias} + R_{wv}^{st})$ and R_{wv}^c are in parallel it is often convenient to express their combined resistance as the leaf resistance for water vapor, R_{wv}^{leaf} :

$$R_{wv}^{leaf} = \frac{(R_{wv}^{ias} + R_{wv}^{st})(R_{wv}^c)}{R_{wv}^{ias} + R_{wv}^{st} + R_{wv}^c} \quad (7.8)$$

When the stomates are open, the cuticular resistance (R_{wv}^c) is generally much larger than the resistance in parallel with it, $R_{wv}^{ias} + R_{wv}^{st}$. In that case, the leaf resistance as given by Equation 7.8 is approximately equal to $R_{wv}^{ias} + R_{wv}^{st}$.

The leaf resistance is in series with that of the unstirred air layer, R_{wv}^a . Thus the total resistance for the flow of water vapor from the cell walls of mesophyll cells to the turbulent air surrounding the plant (R_{wv}^{total}) is equal to $R_{wv}^{leaf} + R_{wv}^a$ (cf. Fig. 7.2). However, we now must face the complication created by the presence of two leaf surfaces which represent parallel pathways for the diffusion of water vapor from the interior of the leaf to the surrounding turbulent air. We will represent the leaf resistance for the pathway through the upper surface by R_{wv}^{leafu} and that for the lower surface by R_{wv}^{leafl} . Each of these resistances is in series with that of an unstirred air layer, R_{wv}^{au} and R_{wv}^{al} for the upper and the lower surfaces, respectively. The parallel arrangement of the pathways through the upper and lower surfaces of a leaf leads to the following comprehensive expression for the

total resistance to the diffusion of water vapor from a leaf:

$$R_{wv}^{total} = \frac{(R_{wv}^{leaf_u} + R_{wv}^{a_u})(R_{wv}^{leaf_l} + R_{wv}^{a_l})}{R_{wv}^{leaf_u} + R_{wv}^{a_u} + R_{wv}^{leaf_l} + R_{wv}^{a_l}} \quad (7.9)$$

Values of resistances. We generally assume that the average thicknesses of the unstirred layers are the same on the two sides of a leaf, in which case $R_{wv}^{a_u}$ in Equation 7.9 is equal to $R_{wv}^{a_l}$. However, this does not simplify our analysis very much, except in certain special cases. If $R_{wv}^{leaf_u} + R_{wv}^{a_u}$ is much greater than is $R_{wv}^{leaf_l} + R_{wv}^{a_l}$, the diffusion of water vapor is mainly out through the lower surface of the leaf. In this case, the total resistance for water vapor diffusion from the cell walls of mesophyll cells to the turbulent air surrounding the leaf (R_{wv}^{total}) is approximately equal to $R_{wv}^{leaf_l} + R_{wv}^{a_l}$. Finally, let us consider the symmetrical case where $R_{wv}^{leaf_u}$ equals $R_{wv}^{leaf_l}$ and the unstirred air layers are of equal thickness, i.e., $R_{wv}^{a_u} = R_{wv}^{a_l}$. For this symmetrical case, Equation 7.9 indicates that the total resistance equals $(R_{wv}^{leaf_l} + R_{wv}^{a_l})^2 / 2(R_{wv}^{leaf_l} + R_{wv}^{a_l})$, which is $(R_{wv}^{leaf_l} + R_{wv}^{a_l})/2$. Since water vapor can move equally well across the upper or lower surface of the leaf in this case, the total resistance of the leaf plus its unstirred air layers for the diffusion of water vapor (R_{wv}^{total}) is half the value for one of the surfaces by itself.

We will now indicate certain general patterns for $R_{wv}^{leaf_l}$ and $R_{wv}^{leaf_u}$. Leaves of most deciduous trees have very few stomates on their upper surfaces, so that R_{wv}^{total} is approximately equal to $R_{wv}^{leaf_l} + R_{wv}^{a_l}$ for them. For some monocots—e.g., grasses such as oats, barley, wheat, and corn—there is a fairly equal distribution of stomates on the two surfaces of the leaves. Thus, for certain grasses we could let R_{wv}^{total} equal $(R_{wv}^{leaf_l} + R_{wv}^{a_l})/2$. For many other plants there are two to four times more stomates on the lower surface of the leaves than on the upper one, with the result that A^s/A is considerably larger for the lower surface; for such leaves, $R_{wv}^{leaf_l}$ is correspondingly smaller than is $R_{wv}^{leaf_u}$. In these intermediate cases it may be necessary to use the rather cumbersome expression for R_{wv}^{total} given by Equation 7.9.

Next, we will examine some representative values for the various resistances encountered by water vapor as it diffuses out of leaves. The resistance of the thin waxy layer covering the cell walls of mesophyll cells is estimated to be 0.1 to 0.2 sec/cm for mesophytes, but may be as much as 2 sec/cm for some xerophytes, where cutinization of the mesophyll cells is appreciable. As we mentioned above, we will include this resistance plus that of the cell wall pores in the resistance of the intercellular air spaces. For crop plants such as beet, spinach, tomato, and pea, the overall R_{wv}^{ias} is generally 0.1

to 0.5 sec/cm, while R_{wv}^{st} usually ranges from 0.4 to 2 sec/cm for open stomates. Both of these resistances tend to be somewhat higher for many other agricultural crops, but R_{wv}^{leaf} is still only 1 to 5 sec/cm for most cultivated plants. For the large leaves of many deciduous trees, R_{wv}^{st} is 3 to 10 sec/cm, while the stomatal resistance (plus that of the stomatal cavities) is often from 10 to 30 sec/cm for conifer needles. R_{wv}^{leaf} is generally 2 to 10 sec/cm for other mesophytes, and 5 to 20 sec/cm for xerophytes. Of course, as the stomates close, R_{wv}^{st} and hence R_{wv}^{leaf} increase from the values cited here for open stomates. Xerophytes also tend to have higher cuticular resistances than mesophytes, viz., R_{wv}^c is usually 20 to 80 sec/cm for mesophytes, and 100 to over 200 sec/cm for xerophytes (see Cowan and Milthorpe as well as Gates 1968).

We will conclude this section with a simplified expression which is often adequate for describing the total resistance to diffusion of water vapor from the sites of evaporation (the mesophyll cell walls) to the turbulent air surrounding a leaf. We will restrict our attention to that situation in which nearly all the water vapor diffusing from the leaf moves out across the lower epidermis and when cuticular transpiration is negligible. By Equations 7.8 and 7.9 the total resistance then is

$$\begin{aligned} R_{wv}^{total} &\cong R_{wv}^{leaf} + R_{wv}^{ai} \\ &\cong R_{wv}^{ias} + R_{wv}^{st} + R_{wv}^{ai} \\ &= \frac{1}{D_{wv}} \left(\delta^{ias} + \frac{d^{st} + r^{st}}{na^{st}} + \delta^a \right) \end{aligned} \quad (7.10)$$

where Equations 7.2, 7.5, and 7.6 have been used to obtain the bottom line.

When diffusion is described by a one-dimensional form of Fick's first law, Equation 7.10 indicates that each part of the pathway contributes its own effective length influencing the movement of water vapor out of the leaf. For instance, the effective length of the unstirred boundary layer is its thickness, δ^a . The distance δ^{ias} includes the effective length of the cell wall pores, the effective length of the waxy layer on the mesophyll cells, and the constriction by the presence of mesophyll cells on the region available for diffusion of gases within a leaf (see p. 311). A similar constricting effect greatly increases the effective length of the stomatal pores over the value of $d^{st} + r^{st}$. Specifically, Equation 7.10 indicates that the effective length of the stomatal pores is $(d^{st} + r^{st})/(na^{st})$, which is considerably greater than $d^{st} + r^{st}$ since na^{st} is much less than one (recall that $na^{st} = A^{st}/A$, p. 309, so that na^{st} is a dimensionless number indicating the fraction of the surface area occupied by stomatal pores). The large effective

length of the stomatal pores caused by the factor $1/na^{st}$ is an alternative way of viewing the constricting effect of the stomates.

If we need to incorporate yet another series resistance for gaseous diffusion, we can include the effective length of the additional part of the pathway within the parentheses in Equation 7.10. For instance, the stomates in some xerophytes and conifers occur sunken beneath the leaf surface in cavities or crypts. We can estimate the effective length of this additional part of the pathway by using appropriate geometrical approximations. The effective length divided by the diffusion coefficient gives us the resistance directly. Also, many leaves are covered by epidermal "hairs," which can be unicellular projections of the epidermal cells or appended multicellular arrays with the cells often occurring in a row. Such *trichomes* cause an additional unstirred air layer to be held next to the leaf surface. The thickness of this layer may equal the average distance that the hairs project perpendicularly away from the leaf surface (more than 1 mm for some leaves). This length can be added within the parenthesis in Equation 7.10 in order to calculate the overall resistance. Also, the extra resistance for water vapor diffusion caused by the hairs can be separately calculated as the thickness of the additional unstirred layer divided by D_{wv} . Some water may also evaporate from the surfaces of the epidermal hairs; this evaporation can lead to a substantial complication in the analysis of water vapor fluxes for leaves with numerous trichomes.

Leaf water vapor concentrations. As we can see from Equation 7.1 ($J_j = \Delta c_j / R_j$), the resistances of the various parts of the pathway determine the drops in concentration across each component when the flux is constant. Here we will apply this condition to a specific consideration of water vapor concentrations in a leaf. We will also discuss the important effect of temperature on the water vapor content of air and the consequences this has for choosing an appropriate way of expressing c_{wv} .

We will represent the difference in water vapor concentration across the intercellular air spaces by Δc_{wv}^{ias} , that across the stomatal pores by Δc_{wv}^{st} , and that across the unstirred air layer adjacent to the leaf surface by Δc_{wv}^a . Thus, the overall drop in water vapor concentration from the cell walls of the mesophyll cells to the turbulent air surrounding the leaf (Δc_{wv}^{total}) is

$$\begin{aligned}\Delta c_{wv}^{total} &= c_{wv}^m - c_{wv}^{ta} \\ &= \Delta c_{wv}^{ias} + \Delta c_{wv}^{st} + \Delta c_{wv}^a\end{aligned}\quad (7.11)$$

where c_{wv}^m is the concentration of water vapor in the cell wall pores of the

mesophyll cells, and c_{wv}^{ta} is its value in the turbulent air surrounding the leaf. We also note that $\Delta c_{wv}^{ias} + \Delta c_{wv}^{st}$ is equal to Δc_{wv}^c , the difference in water vapor concentration from the cell walls of epidermal cells across the cuticle to the leaf surface.

The rate of water vapor diffusion per unit leaf area (J_{wv}) is equal to the difference in water vapor concentration divided by the resistance across which Δc_{wv} occurs ($J_j = \Delta c_j / R_j$, Eq. 7.1). In the steady state, when the flux of water vapor and the resistance of each component are constant, this relation holds both for the overall pathway and any individual segment of it. Some water evaporates from the cell walls of mesophyll cells along that part of the pathway within the leaf, and so J_{wv} is actually not constant in the intercellular air spaces (the guard cells are generally extensively cutinized on the surfaces facing the stomatal pore and so very little water evaporates from such cell walls). For simplicity, however, we generally assume that J_{wv} is unchanging from the mesophyll cell walls all the way out to the turbulent air outside the leaf. When water vapor moves out mainly across the lower epidermis of the leaf and when cuticular transpiration is negligible, we then obtain the following relations in the steady state:

$$J_{wv} = \frac{\Delta c_{wv}^{total}}{R_{wv}^{total}} = \frac{\Delta c_{wv}^{ias}}{R_{wv}^{ias}} = \frac{\Delta c_{wv}^{st}}{R_{wv}^{st}} = \frac{\Delta c_{wv}^a}{R_{wv}^a} \quad (7.12)$$

Equation 7.12 indicates that the drop in water vapor concentration across a particular part of the pathway is proportional to the series resistance of that segment when J_{wv} is constant. For instance, in the steady state a relatively large Δc_{wv} is expected across the stomates, since they have a relatively large resistance.

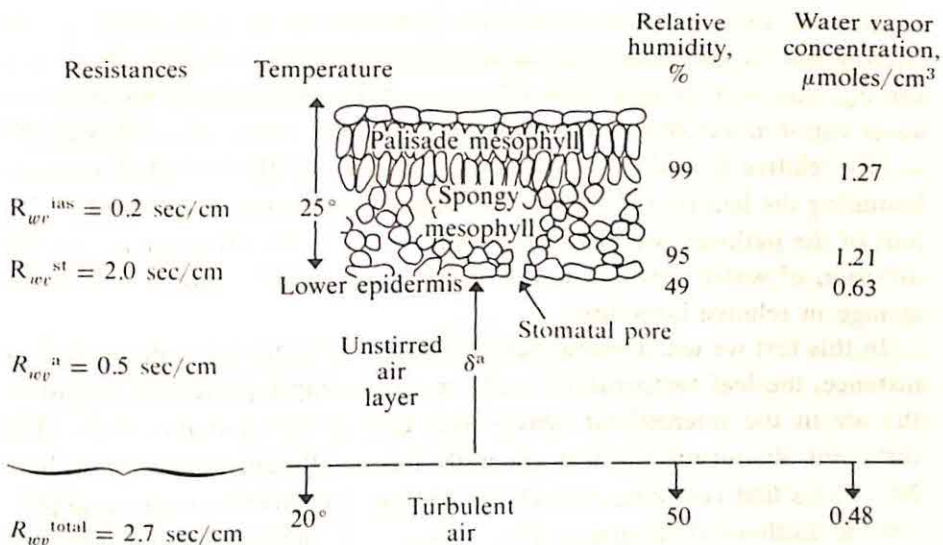
How should we here express the concentration of water vapor for Fick's first law? In Chapter 2 we expressed the water vapor content of air in terms of the relative humidity. By Equation 2.20, the % relative humidity equals $100 P_{wv} / P_{wv}^*$, where P_{wv} is the actual partial pressure of water vapor and P_{wv}^* is the saturation value. As we noted on p. 80, P_{wv}^* is very dependent on temperature, e.g., it is 23.3 mbars at 20° and 42.4 mbars at 30° (App. II). Hence, air saturated with water vapor at 20° has a relative humidity of $(23.3/42.4)(100)$, or only 55%, when heated at constant pressure to 30°. Since air and leaf temperatures are generally not the same, differences in relative humidity are clearly not a suitable measure of the driving force leading to the diffusion of water vapor out of a leaf. However, differences in water vapor concentration (Δc_{wv}) are appropriate for use in gas flux equations derived from Fick's first law of diffusion.

The quantity c_{wv} is sometimes referred to as the *absolute* humidity. Its value in the turbulent air generally does not change very much during the day unless there is precipitation or other marked changes in the weather, while the *relative* humidity can vary greatly as the air temperature changes. Most data for c_{wv} are expressed as mass of water/unit volume of air, therefore we will use $\mu\text{g}/\text{cm}^3$ (numerically equal to g/m^3) as one of our units for water vapor. A unit for c_{wv} which is often more appropriate is $\mu\text{moles}/\text{cm}^3$, and we will use it also. The saturation concentrations of water vapor in air at various temperatures are presented in Appendix II in $\mu\text{g}/\text{cm}^3$ and $\mu\text{moles}/\text{cm}^3$.

Examples of relative humidity and c_{wv} in leaves. We can now calculate the actual relative humidities and concentrations of water vapor in different parts of the transpiration pathway. We will suppose that the leaf temperature is 25° and the turbulent air surrounding the leaf is at 20° with a relative humidity of 50% (Fig. 7.3).

We will begin by assuming that the water vapor in the pores of the cell walls of the mesophyll cells is in equilibrium with the water in the cell wall and that $\Psi^{\text{cell wall}}$ is -8 bars. By Equation 2.21 [$\Psi_{wv} = (RT/\bar{V}_w) \ln (\% \text{ relative humidity}/100) + \rho_w gh$, where $RT/\bar{V}_w$ is 1373 bars at 25° , App. III], we calculate that $\ln (\% \text{ relative humidity}/100)$ for the cell wall pores of the mesophyll cells is $(-8 \text{ bars})/(1373 \text{ bars})$, or -0.006 , and so the relative humidity will be 99.4%. In other words, the air in the pores of the mesophyll cell walls is nearly saturated with water vapor. Using the data in Appendix II ($c_{wv}^* = 23.0 \mu\text{g}/\text{cm}^3$ or $1.28 \mu\text{moles}/\text{cm}^3$ at 25°), 99.4% relative humidity at 25° corresponds to a water vapor concentration (c_{wv}^m) of $(0.994)(23.0)$, or $22.9 \mu\text{g}/\text{cm}^3$, which is $1.27 \mu\text{moles}/\text{cm}^3$. In the turbulent air surrounding the leaf (20° , 50% relative humidity), data in Appendix II ($c_{wv}^* = 17.3 \mu\text{g}/\text{cm}^3$ or $0.96 \mu\text{mole}/\text{cm}^3$ at 20°) indicate that c_{wv}^{ta} is $(0.50)(17.3)$, or $8.7 \mu\text{g}$ of $\text{H}_2\text{O}/\text{cm}^3$, which is $0.48 \mu\text{mole}/\text{cm}^3$. Thus, the difference in water vapor concentration from the mesophyll cells to the turbulent air ($\Delta c_{wv}^{\text{total}} = c_{wv}^m - c_{wv}^{\text{ta}}$, Eq. 7.11) is here $22.9 - 8.7$, or $14.2 \mu\text{g}/\text{cm}^3$, which is the same as $1.27 - 0.48$, or $0.79 \mu\text{mole}/\text{cm}^3$.

Equation 7.11 indicates that $\Delta c_{wv}^{\text{total}}$ is the sum of three components, $\Delta c_{wv}^{\text{ias}}$, $\Delta c_{wv}^{\text{st}}$, and Δc_{wv}^{a} . The magnitude of each of these differences in water vapor concentration is proportional to the particular value of the resistance across which the drop occurs, i.e., $\Delta c_{wv}^x / \Delta c_{wv}^{\text{total}} = R_{wv}^x / R_{wv}^{\text{total}}$, where x refers to any series component of the pathway (cf. Eq. 7.12). For definiteness, we will let R_{wv}^{ias} be $0.2 \text{ sec}/\text{cm}$, R_{wv}^{st} be $2.0 \text{ sec}/\text{cm}$, and R_{wv}^{a} be $0.5 \text{ sec}/\text{cm}$ (see Fig. 7.3). Hence, the overall resistance to the diffusion of water vapor from the pores of the cell walls of mesophyll cells to the turbulent air

**Figure 7.3**

Representative values of quantities influencing the diffusion of water vapor out of a leaf.

surrounding the leaf (R_{wv}^{total}) is $0.2 + 2.0 + 0.5$, or 2.7 sec/cm . As we can see from Equation 7.12, Δc_{wv}^{ias} is equal to $(R_{wv}^{ias}/R_{wv}^{total}) \Delta c_{wv}^{total}$, which in the present case is $[(0.2 \text{ sec/cm})/(2.7 \text{ sec/cm})](0.79 \mu\text{mole/cm}^3)$, or $0.06 \mu\text{mole/cm}^3$. Likewise, Δc_{wv}^{st} is $(2.0/2.7)(0.79)$, or $0.58 \mu\text{mole/cm}^3$, while Δc_{wv}^a is $(0.5/2.7)(0.79)$, or $0.15 \mu\text{mole H}_2\text{O/cm}^3$. Hence, the largest drop in water vapor concentration, $0.58 \mu\text{mole/cm}^3$, occurs here across the stomatal pores, which contribute the largest resistance to R_{wv}^{total} . The actual concentrations of water vapor along the transpiration pathway for this example are summarized in Figure 7.3.

Since c_{wv}^m is $1.27 \mu\text{moles/cm}^3$ and Δc_{wv}^{ias} is $0.06 \mu\text{mole/cm}^3$, the water vapor concentration on the inner side of the stomatal pores must be $1.27 - 0.06$, or $1.21 \mu\text{moles/cm}^3$. The saturation concentration of water vapor is $1.28 \mu\text{moles/cm}^3$ at 25° (App. II), and so the air on the inner side of the stomates has a relative humidity of $[(1.21 \mu\text{moles/cm}^3)/(1.28 \mu\text{moles/cm}^3)](100)$, or 95% (see Fig. 7.3). Therefore, a gradient in relative humidity from 99% in the cell walls of mesophyll cells to 95% at the inner side of the stomates is necessary here for the diffusion of water vapor across the intercellular air spaces. Across the stomatal pores, which we also assume are at 25° , Δc_{wv}^{st} is $0.58 \mu\text{mole/cm}^3$ and so the relative humidity drops from 95% to $[(1.21 - 0.58)/(1.28)](100)$, or 49%, the value it has at the leaf surface. Although we should view these numbers only as approximate guides, they

do indicate that the drop in relative humidity is not very great in the intercellular air spaces and that an appreciable gradient in relative humidity and c_{wv} can exist along a stomatal pore. After crossing the stomatal pores, water vapor moves *energetically downhill* from 49% relative humidity at 25° to 50% relative humidity at 20° as it diffuses across the unstirred air layer bounding the leaf (Fig. 7.3). Since a temperature change is involved in this part of the pathway, we obviously need to express the driving force for the diffusion of water vapor in terms of the concentration difference, not the change in relative humidity.

In this text we will assume that the leaf has a uniform temperature. For instance, the leaf temperature is 25° in the example presented, including the air in the intercellular spaces and that in the stomatal pores. The turbulent air outside the leaf generally has a different temperature, here 20°. Fick's first law (e.g., $J_j = D_j \Delta c_j / \Delta x$, Eq. 7.1) strictly speaking applies only to isothermal situations. For instance, D_j depends on the absolute temperature ($D_j = u_j RT$, p. 105), so that $\Delta(D_j c_j)$ may be nonzero and lead to a flux even when Δc_j is zero. Fortunately, even when there are temperature differences between leaves and the turbulent air, Fick's first law in the form of Equation 7.1 has proved adequate for describing the fluxes of H₂O and CO₂, our primary concern in this chapter. In a later section we will show how the temperature difference across the unstirred air layer bounding a leaf leads to a heat flux.

Fluxes of water vapor. Since we have already presented the resistances and the concentration drops involved, it is a straightforward matter to calculate the flux of water vapor out of a transpiring leaf. We will employ the values used in the previous section, viz., a R_{wv}^{total} of 2.7 sec/cm and a $\Delta c_{wv}^{\text{total}}$ of 14.2 $\mu\text{g}/\text{cm}^3$, which is 0.79 $\mu\text{mole}/\text{cm}^3$. From Fick's first law of diffusion as expressed by Equation 7.12 ($J_{wv} = \Delta c_{wv}^{\text{total}} / R_{wv}^{\text{total}}$), we calculate that the flux of water vapor equals $(14.2 \mu\text{g}/\text{cm}^3) / (2.7 \text{ sec}/\text{cm})$, which is 5.3 $\mu\text{g}/\text{cm}^2\text{-sec}$; or, equivalently, J_{wv} equals $(0.79 \mu\text{mole}/\text{cm}^3) / (2.7 \text{ sec}/\text{cm})$, which is 0.29 $\mu\text{mole}/\text{cm}^2\text{-sec}$. Thus, a flux of water vapor expressed as rate of movement of mass per unit area is associated with c_{wv} in mass/unit volume, while a molar flux corresponds to c_{wv} expressed in moles/unit volume.

So far, for simplicity, we have considered the movement of water vapor across only the lower surface of a leaf, ignoring cuticular transpiration. The cuticular transpiration (flux = $\Delta c_{wv}^c / R_{wv}^c$) is generally small compared with the transpiration through open stomates in parallel with it, i.e., Δc_{wv}^c is nearly the same as $\Delta c_{wv}^{\text{st}}$, while R_{wv}^c is here much greater than R_{wv}^{st} . To give the overall rate of water vapor diffusing out of the leaves, we could

scale up J_{wv} calculated for the lower surface by an appropriate factor (the reciprocal of the fraction of transpiration through the lower surface). For instance, about 70% of the water loss in transpiration might be through the lower surface for a representative mesophyte. The J_{wv} of $5.3 \mu\text{g}/\text{cm}^2\text{-sec}$ which we calculated in the previous paragraph would then become $(1/0.70)(5.3)$, or $7.6 \mu\text{g}/\text{cm}^2\text{-sec}$, while the molar flux would be $(1/0.70)(0.29)$, or $0.41 \mu\text{mole}/\text{cm}^2\text{-sec}$ when both leaf surfaces are considered, i.e., $J_{wv}^1 = 0.70 \times \text{overall } J_{wv}$ implies that overall $J_{wv} = J_{wv}^1/0.70$. Alternatively, we could use the actual R_{wv}^{total} as given by Equation 7.9 which considers the two leaf surfaces in parallel. The $\Delta c_{wv}^{\text{total}}$ divided by this R_{wv}^{total} would give J_{wv} through both surfaces but expressed per unit area of one side of the leaf, i.e., the flux would still be expressed on the same basis as the other fluxes in this chapter.

For most mesophytes under the above conditions ($T^{\text{leaf}} = 25^\circ$, $T^{\text{ta}} = 20^\circ$, relative humidity $^{\text{ta}} = 50\%$), J_{wv} for open stomates is from 2 to $10 \mu\text{g}/\text{cm}^2\text{-sec}$, which is about 0.1 to $0.5 \mu\text{mole}/\text{cm}^2\text{-sec}$. Therefore, the example we have used in the previous paragraph (overall $J_{wv} = 7.6 \mu\text{g}/\text{cm}^2\text{-sec}$ or $0.41 \mu\text{mole}/\text{cm}^2\text{-sec}$) is a slightly above average transpiration rate for a mesophyte. Transpiration rates of xerophytes tend to be lower than those of mesophytes, e.g., J_{wv} for xerophytes is often 0.5 to $3 \mu\text{g}/\text{cm}^2\text{-sec}$ or 0.03 to $0.2 \mu\text{mole}/\text{cm}^2\text{-sec}$ for open stomates under the above conditions (see Cowan and Milthorpe as well as Kramer). Unfortunately, many systems of units are used for the rates of water vapor leaving unit area of leaves. The conversion factors for the more common systems of expressing transpiration are summarized in Table 7.2.

Stomatal control of J_{wv} . Let us now reconsider the values of the various resistances affecting the diffusion of water vapor through the intercellular air spaces, out the stomates, and across the unstirred layer at the leaf surface. R_{wv}^{ias} is relatively small and R_{wv}^{a} rarely exceeds $2 \text{ sec}/\text{cm}$, while R_{wv}^{st} continuously increases as the stomates close. Since R_{wv}^{total} is often approximately equal to $R_{wv}^{\text{ias}} + R_{wv}^{\text{st}} + R_{wv}^{\text{a}}$ (Eq. 7.10), the ultimate control for reducing transpiration rests with the stomates, not with the unstirred air layer or the intercellular air spaces. When R_{wv}^{st} is a few times larger than R_{wv}^{a} , as often occurs under field conditions, moderate changes in the ambient wind velocity have relatively little effect on R_{wv}^{total} . In such cases a wind increase does not increase transpiration significantly (recall that $R_{wv}^{\text{a}} = \delta^{\text{a}}/D_{wv}$, Eq. 7.2, where $\delta^{\text{a}}_{(\text{cm})} \cong 0.4 \sqrt{l^{\text{leaf}}_{(\text{cm})}/v^{\text{wind}}_{(\text{cm}/\text{sec})}}$, Eq. 7.3). Conversely, leaf size and ambient wind velocity tend to have a major influence on R_{wv}^{total} and transpiration only when R_{wv}^{st} is relatively small compared with R_{wv}^{a} .

Table 7.2

Conversion factors for some of the more common units used in expressing wind velocities, transpiration, CO₂ concentrations, photosynthesis, as well as heat and energy fluxes. The actual numbers were determined using quantities in Appendix III. To convert from one set to another, a quantity expressed in the units in the left column should be multiplied by the factor in the column of the desired units. For example, to convert wind velocity from cm/sec to m/sec, multiply by the factor 0.0100.

Wind Velocity Units	cm/sec	m/sec	km/hr	miles/hr
cm/sec	—	0.01000	0.0360	0.0224
m/sec	100.0	—	3.60	2.24
km/hr	27.8	0.278	—	0.621
miles/hr	44.7	0.447	1.609	—

Transpiration Units	μmoles/cm ² -sec	μg/cm ² -sec
μmoles H ₂ O/cm ² -sec	—	18.02
μg H ₂ O/cm ² -sec	0.0555	—
μg H ₂ O/cm ² -min	9.25×10^{-4}	0.01667
mg H ₂ O/dm ² -min	9.25×10^{-3}	0.1667
g H ₂ O/dm ² -hr	0.1542	2.78

CO ₂ Concentrations	nmoles/cm ³	ng/cm ³ (mg/m ³)	μliters/liter* (ppm by volume)
nmoles CO ₂ /cm ³	—	44.0	24.4
ng CO ₂ /cm ³ (mg CO ₂ /m ³)	0.0227	—	0.554
μliters CO ₂ /liter* (ppm CO ₂ by volume)	0.0410	1.806	—

Photosynthesis Units	nmoles CO ₂ /cm ² -sec
ng CO ₂ /cm ² -sec	0.0227
kg CO ₂ /m ² -sec	2.27×10^6
mg CO ₂ /dm ² -hr (kg CO ₂ /hectare-hr)	0.0631
kg carbohydrate/hectare-hr	0.092
mm ³ CO ₂ /cm ² -hr*	0.0114

Heat and Energy Flux Units	cal/cm ² -min	watts/cm ² (joules/cm ² -sec)	ergs/cm ² -sec
cal/cm ² -min	—	0.0697	6.97×10^5
watts/cm ² (joules/cm ² -sec)	14.34	—	1.000×10^7
watts/m ² (joules/m ² -sec)	0.001434	1.000×10^{-4}	1000
ergs/cm ² -sec	1.434×10^{-6}	1.000×10^{-7}	—

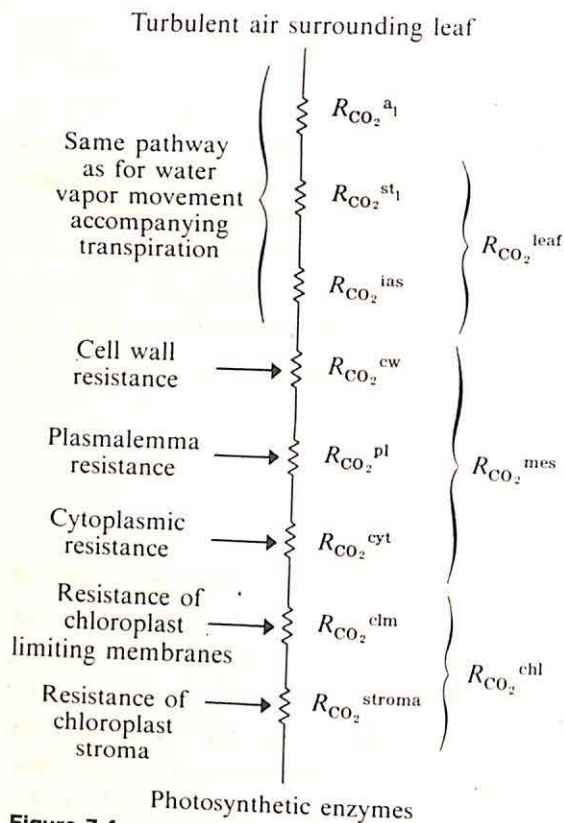
*To convert a volume/volume number like ppm to a mole/volume one (or simply to convert volume of gas to moles), we need to know the temperature and the pressure; an air temperature of 20° and a pressure of 1 bar were used here. To adjust for other temperatures and pressures, the perfect gas law must be employed, e.g., 1 ppm of CO₂ at temperature T_x in °K and pressure P_x in bars is equal to $0.0410 (293.15/T_x)(P_x)$ nmoles/cm³.

Stomates tend to close as a plant wilts. As wilting proceeds, R_{wv}^{st} might increase from 1.0 sec/cm up to 20 sec/cm due to the stomatal closure. If R_{wv}^a were 0.5 sec/cm and R_{wv}^{ias} were 0.2 sec/cm, wilting would then be accompanied by a change of R_{wv}^{total} from 1.7 sec/cm to 20.7 sec/cm, and a corresponding decrease in J_{wv} to $[(1.7 \text{ sec/cm})/(20.7 \text{ sec/cm})](100)$, or only 8% of its former value. Such a decrease in transpiration caused by stomatal closure is a common response of plants to water stress, since R_{wv}^{st} tends to increase when water potentials in the soil and the leaf fall. When the stomates close tightly, R_{wv}^{st} can exceed 100 sec/cm, in which case cuticular transpiration would become a relatively important pathway for the loss of water vapor from the leaves.

CO₂ fluxes accompanying photosynthesis

We will next consider the main function of a leaf, photosynthesis, in terms of the resistances encountered by carbon dioxide as it diffuses from the turbulent air, across the unstirred layers next to a leaf, through the stomates, across the intercellular air spaces, into the mesophyll cells, and eventually into the chloroplasts. The situation is obviously more complicated than the analogous one of the movement of water vapor during transpiration, since CO₂ must not only diffuse across the same resistances encountered by water vapor moving in the opposite direction, but must also cross the cell wall of a mesophyll cell, the plasmalemma, part of the cytoplasm, the membranes surrounding a chloroplast, and some of the chloroplast stroma. We will consider two important cellular processes which evolve CO₂, respiration and photorespiration. To conclude this section we will estimate J_{CO_2} , the apparent (net) rate of photosynthesis by a leaf.

Resistance network. Figure 7.4 illustrates the various resistances affecting CO₂ as it diffuses from the turbulent air surrounding a leaf up to the sites in the chloroplasts where it is incorporated into photosynthetic products. For simplicity, we will restrict our attention to the case where CO₂ diffuses into the leaf mainly across the lower epidermis. When the parallel pathways through the upper and lower surfaces of a leaf are both important, we can readily modify our equations to handle the reduction in resistance encountered between the turbulent air surrounding the leaf and the cell walls of mesophyll cells (cf. Eq. 7.9). We will also ignore the cuticular path for CO₂ entry into the leaf for the same sort of reason we neglected this part of the pathway when discussing transpiration, viz., $R_{CO_2}^c$ is generally considerably greater than $R_{CO_2}^{ias} + R_{CO_2}^{st}$, the resistance in parallel with it.

**Figure 7.4**

Principal resistances involved in the movement of CO_2 from the turbulent air surrounding a leaf, across the lower epidermis, up to the enzymes involved in the fixation of CO_2 into photosynthetic products in the chloroplasts of mesophyll cells.

The first three resistances encountered by CO_2 entering a leaf through the lower epidermis ($R_{CO_2}^{a_1}$, $R_{CO_2}^{st_1}$, and $R_{CO_2}^{ias}$) have analogs in the case of transpiration. We can thus transfer Equation 7.10 to our present discussion, changing only the subscripts:

$$R_{CO_2}^{leaf_1} + R_{CO_2}^{a_1} = \frac{1}{D_{CO_2}} \left(\delta_{ias} + \frac{d^{st} + r^{st}}{na^{st}} + \delta^a \right) \quad (7.13)$$

where $R_{CO_2}^{leaf_1}$ is the resistance of the intercellular air spaces plus the stomatal pores of the lower leaf surface to the diffusion of CO_2 . We also

note that $R_{\text{CO}_2}^{\text{leaf}_1} + R_{\text{CO}_2}^{\text{a}_1}$ is analogous to $R_{\text{wv}}^{\text{total}}$, the *total* resistance encountered by water vapor (cf. Fig. 7.4).

The diffusion coefficient for CO₂ (D_{CO_2}) is 0.16 cm²/sec in air at 20°. This is smaller than D_{wv} (0.25 cm²/sec at 20°) because CO₂ molecules are heavier and thus diffuse more slowly than H₂O molecules (cf. p. 18). By Equations 7.10 and 7.13, $(R_{\text{CO}_2}^{\text{leaf}_1} + R_{\text{CO}_2}^{\text{a}_1})/R_{\text{wv}}^{\text{total}}$ is equal to $D_{\text{wv}}/D_{\text{CO}_2}$, which is (0.25 cm²/sec)/(0.16 cm²/sec), or 1.6. Consequently, CO₂ diffusing from the turbulent air up to the cell walls of mesophyll cells encounters 60% more resistance than water vapor diffusing in the opposite direction over this same pathway. For instance, $R_{\text{wv}}^{\text{leaf}_1}$ is generally 1 to 10 sec/cm for mesophytes with open stomates. $R_{\text{CO}_2}^{\text{leaf}_1}$ would then be 2 to 15 sec/cm for such plants, while $R_{\text{CO}_2}^{\text{leaf}_1}$ for xerophytes is usually in the range of 7 to 30 sec/cm when the stomates are open.

As indicated in Figure 7.4, five additional resistances are involved in carbon dioxide flow compared with water vapor movement. The new components of the pathway are the nongaseous parts of the cell wall of a mesophyll cell (resistance = $R_{\text{CO}_2}^{\text{cw}}$), its plasmalemma ($R_{\text{CO}_2}^{\text{pl}}$), and its cytoplasm ($R_{\text{CO}_2}^{\text{cyt}}$), then the chloroplast limiting membranes ($R_{\text{CO}_2}^{\text{clm}}$), and finally the interior of the chloroplasts ($R_{\text{CO}_2}^{\text{stroma}}$). For convenience we will divide these five resistances into two parts, the mesophyll resistance to CO₂, $R_{\text{CO}_2}^{\text{mes}}$, and the chloroplast resistance, $R_{\text{CO}_2}^{\text{chl}}$:

$$R_{\text{CO}_2}^{\text{mes}} = R_{\text{CO}_2}^{\text{cw}} + R_{\text{CO}_2}^{\text{pl}} + R_{\text{CO}_2}^{\text{cyt}} \quad (7.14a)$$

$$R_{\text{CO}_2}^{\text{chl}} = R_{\text{CO}_2}^{\text{clm}} + R_{\text{CO}_2}^{\text{stroma}} \quad (7.14b)$$

Mesophyll area. The area of the cell walls of mesophyll cells across which CO₂ can diffuse is considerably larger than the surface area of the leaf. For the constricting effect caused by the stomates we used A^{st}/A to give the fraction of the leaf surface area occupied by stomatal pores. Here we will use the ratio A^{mes}/A to indicate the *increase* in area available for CO₂ diffusion, where A^{mes} is the total area of the cell walls of mesophyll cells that is exposed to the intercellular air spaces, while A is the area of one side of the same leaf.

Although A^{mes}/A varies with plant species as well as with leaf development, it is generally in the range of 15 to 40 for mesophytes (see El-Sharkawy and Hesketh as well as Esau). We can appreciate the large value of A^{mes}/A by examining Figures 1.2 and 7.3, which indicate that there is a considerable amount of air space and hence exposed cell walls within a leaf, e.g., the palisade mesophyll is generally 10–40% air by volume, while the spongy mesophyll is 50–80% air. Although there is a greater volume of air in the

spongy mesophyll region, the palisade cells usually have a greater total cell wall area exposed to the intercellular air spaces than do spongy mesophyll cells (cf. Fig. 1.2). Xerophytes tend to have a somewhat more highly developed palisade region than do mesophytes (in some cases, the spongy mesophyll cells are even absent in xerophytes), which leads to values of 20 to 70 for A^{mes}/A of many xerophytes.

Most of the exposed cell wall area of the palisade cells actually occurs on their lateral walls. To demonstrate this point, let us represent the palisade cells as cylinders with their axes perpendicular to the leaf surface. If r is the radius and l is the length of a cylinder, then the total area of the ends of a cylinder ($2\pi r^2$) is less than its lateral surface area ($2\pi rl$), since l is greater than r for such palisade cells, e.g., r may be 10 to 20 μm , while l is 60 to 120 μm for representative palisade cells. When such cylinders are packed together to form a layer of palisade cells, nearly the entire surface area of the lateral walls is exposed to the intercellular air spaces. This area and most of that of both ends of a palisade cell are available for the inward diffusion of CO_2 .

The mesophyll region of a leaf can be greatly influenced by the illumination condition under which the leaf develops. Development in a dark or shaded environment can lead to a shade leaf, while differentiation under moderate to high illumination can lead to a sun leaf (see Esau). Sun leaves usually have a higher proportion of palisade cells than shade leaves on the same plant, and more importantly, the palisade cells are generally longer in sun leaves (larger l for the cylinders) than in shade leaves. Consequently, the ratio A^{mes}/A is often at least twice as large in sun leaves compared with shade leaves on the same plant.

The resistance to diffusion of some molecular species across a barrier equals the reciprocal of its permeability coefficient, as we indicated on p. 305. We will let $P_{\text{CO}_2}^j$ be the permeability coefficient for CO_2 diffusion across barrier j . To calculate the resistance of a particular mesophyll or chloroplast component, we must also incorporate the factor A^{mes}/A to allow for the actual area available for diffusion. Since the area of the plasmalemma is about the same as that of the cell wall, and the chloroplasts generally occupy a single layer around the periphery of the cytoplasm (Fig. 1.1), the factor A^{mes}/A will be applied to all the diffusion steps of CO_2 with which we are concerned in mesophyll cells (all 5 individual resistances in Eq. 7.14). In other words, we are imagining for simplicity that the cell wall, the plasmalemma, the cytoplasm, and the chloroplasts are all in layers having equal areas. Thus, the resistance of any of the mesophyll or chloroplast components for CO_2 diffusion ($R_{\text{CO}_2}^j$) is reduced from $1/P_{\text{CO}_2}^j$ by the reciprocal of the

same factor, A^{mes}/A :

$$R_{\text{CO}_2}^j = \frac{1}{A^{\text{mes}}/A} \frac{1}{P_{\text{CO}_2}^j} = \frac{A}{A^{\text{mes}} P_{\text{CO}_2}^j} \\ = \frac{A \Delta x^j}{A^{\text{mes}} D_{\text{CO}_2}^j K_{\text{CO}_2}^j} \quad (7.15)$$

where the second line of this equation follows from the definition of a permeability coefficient ($P_j = D_j K_j / \Delta x$, Eq. 1.9). In particular, Δx^j is the thickness of the j^{th} barrier, $D_{\text{CO}_2}^j$ is the diffusion coefficient of CO₂ in it, while $K_{\text{CO}_2}^j$ is a suitably defined partition coefficient.

Cell wall resistance. Let us begin our discussion of the newly introduced resistances by evaluating the components of $R_{\text{CO}_2}^{\text{cw}}$, the resistance encountered by carbon dioxide as it diffuses through the water-filled interstices of the cell wall from the interface with the intercellular air spaces on one side to the plasmalemma on the other side. We will use Equation 7.15 to describe this resistance, viz., $R_{\text{CO}_2}^{\text{cw}} = A \Delta x^{\text{cw}} / (A^{\text{mes}} D_{\text{CO}_2}^{\text{cw}} K_{\text{CO}_2}^{\text{cw}})$.

The distance across the barrier (Δx^{cw}) is the average thickness of the cell walls of the mesophyll cells. The diffusion coefficient for the gas CO₂ dissolved in water is 1.7×10^{-5} cm²/sec at 20°. However, the effective $D_{\text{CO}_2}^{\text{cw}}$ is probably lower by a factor of three or four, because the water-filled interstices represent slightly less than half of the cell wall and their direction through the cell wall is rather tortuous. For instance, $D_{\text{CO}_2}^{\text{cw}}$ may be about 5×10^{-6} cm²/sec. In addition to moving as the dissolved gas, "CO₂" may also diffuse across the cell wall as H₂CO₃ or HCO₃⁻. The diffusion coefficients of these two species in the cell wall are also most likely about 5×10^{-6} cm²/sec. However, the possible presence of H₂CO₃ and HCO₃⁻ makes the effective concentration of "CO₂" in the cell wall uncertain.

In Chapter 1 we introduced a partition coefficient (p. 28) in order to describe the ratio of the concentrations of some species in two adjacent phases. For instance, $K_{\text{CO}_2} c_{\text{CO}_2}$ could be the actual concentration of CO₂ in some phase where concentrations are difficult to measure, where c_{CO_2} is the readily measured equilibrium concentration of carbon dioxide in an adjacent phase and K_{CO_2} is the partition coefficient. Similarly, we will express every $K_{\text{CO}_2}^j$ in the mesophyll cells as the actual concentration of all forms of CO₂ in component j divided by the concentration of CO₂ in an adjacent air phase at equilibrium, $c_{\text{CO}_2}^j$. A concentration referred to as $c_{\text{CO}_2}^j$ will thus equal the actual concentration in component j divided by $K_{\text{CO}_2}^j$. (This convention allows us to discuss fluxes in a straightforward manner, as CO₂ then diffuses toward regions of lower $c_{\text{CO}_2}^j$, regardless of the actual concentrations and partition coefficients involved.) Here we are interested in the

partitioning of CO_2 between the air in the cell wall pores and the various species of CO_2 in the adjacent water within the cell wall interstices. $K_{\text{CO}_2}^{\text{cw}}$ is thus the actual concentrations of CO_2 , H_2CO_3 , and HCO_3^- in the cell wall water divided by the concentration of CO_2 in air in equilibrium with the cell wall water.

The concentrations of the various forms of " CO_2 " present in an aqueous phase are temperature dependent as well as extremely sensitive to pH.* For instance, the equilibrium concentration of CO_2 dissolved in water divided by that of CO_2 in an adjacent gas phase, $c_{\text{CO}_2}/c_{\text{CO}_2}^{\text{air}}$, is 1.19 at 10° , 0.88 at 20° , 0.67 at 30° , and 0.53 at 40° . This partition coefficient is itself not very pH dependent, but the equilibrium concentration of HCO_3^- in water relative to that of dissolved CO_2 is markedly affected by pH. In particular, CO_2 dissolved in an aqueous solution may interact with OH^- to form bicarbonate, which then associates with H^+ to form H_2CO_3 : $\text{CO}_2 + \text{OH}^- + \text{H}^+ \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \rightleftharpoons \text{H}_2\text{CO}_3$. Alternatively, CO_2 in water might form H_2CO_3 , which then dissociates to HCO_3^- and H^+ . The interconversions of CO_2 and H_2CO_3 are actually relatively slow unless a suitable catalyst, such as the enzyme carbonic anhydrase, is present (see Forster *et al.*, as well as Maren). Since H^+ is involved in these reactions, its concentration, i.e., the pH, will affect the amount of HCO_3^- in solution, which in turn depends on the CO_2 concentration—the equilibrium concentration of H_2CO_3 is only about $1/400$ of that of the dissolved CO_2 , and so our main concern will be with CO_2 and HCO_3^- .

At 20° , $(c_{\text{CO}_2} + c_{\text{H}_2\text{CO}_3} + c_{\text{HCO}_3^-})/c_{\text{CO}_2}^{\text{air}}$ (our definition for $K_{\text{CO}_2}^{\text{cw}}$) equals 0.88 at pH 4, 0.91 at pH 5, 1.23 at pH 6, 4.4 at pH 7, and about 35 at pH 8. These equilibrium values are affected by temperature in approximately the same way as the partition coefficient $c_{\text{CO}_2}/c_{\text{CO}_2}^{\text{air}}$ cited above, e.g., going from 20° to 30° they change by about $(0.67)/(0.88)$, or 0.76, which is a decrease of 24%. Although the pH in the cell walls of mesophyll cells within a leaf is not known with certainty, it is probably under 6. Thus, at usual leaf temperatures $K_{\text{CO}_2}^{\text{cw}}$ will be close to unity, the value we will use for calculation.

Let us now estimate a value for $R_{\text{CO}_2}^{\text{cw}}$. We will assume that the thickness of the cell walls (Δx^{cw}) is $1.0 \mu\text{m}$, that the diffusion coefficient in the cell walls for solutes like CO_2 or HCO_3^- ($D_{\text{CO}_2}^{\text{cw}}$) is $5 \times 10^{-6} \text{ cm}^2/\text{sec}$, and that $K_{\text{CO}_2}^{\text{cw}}$ is 1.0. The magnitude of $R_{\text{CO}_2}^{\text{cw}}$ also depends on the relative surface area of the mesophyll cells compared to the leaf, a quantity which varies

* The concentrations also depend on the presence of other solutes, but this effect is generally small for the cell wall water.

considerably. We will let A^{mes}/A be 20, a representative value for mesophytes. Using Equation 7.15, $R_{\text{CO}_2}^{\text{cw}} = A\Delta x^{\text{cw}}/(A^{\text{mes}}D_{\text{CO}_2}^{\text{cw}}K_{\text{CO}_2}^{\text{cw}})$, we then calculate that the effective resistance of the cell walls to the diffusion of CO₂ is $(1/20)(1.0 \times 10^{-4} \text{ cm})/[(5 \times 10^{-6} \text{ cm}^2/\text{sec})(1.0)]$, or 1 sec/cm. This is a rather small value for a CO₂ resistance and indicates that the cell walls of the mesophyll cells generally do not represent a major barrier to the diffusion of the various species of CO₂.

Plasmalemma resistance. Let us now examine $R_{\text{CO}_2}^{\text{pl}}$, the effective resistance of the plasmalemmas of mesophyll cells to diffusion of the various species of CO₂. Although we do not know the actual permeability coefficients of the plasmalemmas of mesophyll cells for CO₂ and HCO₃⁻, we expect them to be much lower for a charged species like HCO₃⁻ (cf. p. 32). For instance, $P_{\text{HCO}_3^-}^{\text{pl}}$ might be about 10⁻⁶ cm/sec. On the other hand, CO₂ is a small neutral linear molecule which enters cells extremely easily (see Forster *et al.*). The permeability coefficient of CO₂ entering plant cells is probably at least 10⁻² cm/sec, which is in the same range as $P_{\text{H}_2\text{O}}^{\text{pl}}$ (p. 32). Using Equation 7.15, $R_{\text{CO}_2}^j = (A/A^{\text{mes}})/P_{\text{CO}_2}^j$, and a value of 20 for A^{mes}/A , we calculate that $R_{\text{CO}_2}^{\text{pl}}$ would be $(1/20)/(10^{-2} \text{ cm/sec})$, or 5 sec/cm, for CO₂. Based on the relative values for the two permeability coefficients, the resistance to the diffusion of HCO₃⁻ across the plasmalemma could be about 10⁴ times higher, namely 5 × 10⁴ sec/cm. Because of the extremely high resistance value for HCO₃⁻, we conclude that bicarbonate does not diffuse across the plasmalemma at the rates necessary to sustain photosynthesis. The resistance value calculated for CO₂ (about 5 sec/cm) is only moderate, and suggests that diffusion of CO₂ can be adequate for moving this substrate of photosynthesis across the plasmalemma.

HCO₃⁻ or even CO₂ could be actively transported across the plasmalemma, or perhaps could cross by facilitated diffusion. Facilitated diffusion would act like a low resistance pathway in parallel with the ordinary diffusion pathway and thus would reduce the effective resistance of the plasmalemma to either of these species. Unfortunately, the actual mechanism for CO₂ or HCO₃⁻ movement across the plasmalemmas of mesophyll cells is not known with certainty, although $R_{\text{CO}_2}^{\text{pl}}$ for diffusion of CO₂ is probably low enough to account for the CO₂ fluxes actually observed.

Cytoplasmic resistance. The resistance of the cytoplasm of the mesophyll cells to the diffusion of CO₂ is small, since the chloroplasts are located around the periphery of most mesophyll cells. The average distance from the plasmalemma to the chloroplasts (Δx^{cyt}) is only 0.1 to 0.3 μm . We will use a value of 0.2 μm for Δx^{cyt} in order to estimate $R_{\text{CO}_2}^{\text{cyt}}$ using the bottom line of Equation 7.15.

Due to the presence of a considerable amount of protein, the cytoplasm between the plasmalemma and the chloroplasts occurs in the form of a gel-like matrix. The diffusion coefficient of CO_2 in this region is somewhat less than its value in water at 20° , $1.7 \times 10^{-5} \text{ cm}^2/\text{sec}$. For purposes of calculation we will let $D_{\text{CO}_2}^{\text{cyt}}$ be $1 \times 10^{-5} \text{ cm}^2/\text{sec}$ for the various species of CO_2 diffusing from the plasmalemma to the chloroplasts. The value of $K_{\text{CO}_2}^{\text{cyt}}$ of mesophyll cells is not known, primarily because of the lack of knowledge about cytoplasmic pH. In any case, $K_{\text{CO}_2}^{\text{cyt}}$ cannot be much less than unity, and values greater than 1.0—the magnitude we will assume here—will not change our conclusions about the relative value of $R_{\text{CO}_2}^{\text{cyt}}$. As before, we will let A^{mes}/A be 20, while Δx^{cyt} will be $0.2 \mu\text{m}$. Using Equation 7.15, $R_{\text{CO}_2}^j = A\Delta x^j / (A^{\text{mes}} D_{\text{CO}_2}^j K_{\text{CO}_2}^j)$, we calculate that $R_{\text{CO}_2}^{\text{cyt}}$ is equal to $(1/20)(0.2 \times 10^{-4}) / [(1 \times 10^{-5})(1.0)]$, or only 0.1 sec/cm , a very small value for a resistance to CO_2 diffusion. Thus, the location of the chloroplasts around the periphery of a mesophyll cell is a “good” design in that it causes the resistance of this part of the pathway for CO_2 diffusion to be quite low.

Mesophyll resistance. Let us now compare the measured values of the mesophyll resistance with the magnitudes just calculated for its three components. Most of the measurements of $R_{\text{CO}_2}^{\text{mes}}$ have been rather indirect, but they do indicate that this resistance is generally 2 to 8 sec/cm for mesophytes (see Šetlik; Waggoner, and Zelitch). Based on the estimates of 1 sec/cm for $R_{\text{CO}_2}^{\text{cw}}$, 5 sec/cm for $R_{\text{CO}_2}^{\text{pl}}$, and 0.1 sec/cm for $R_{\text{CO}_2}^{\text{cyt}}$, we would predict a value near 6 sec/cm for $R_{\text{CO}_2}^{\text{mes}}$ ($R_{\text{CO}_2}^{\text{mes}} = R_{\text{CO}_2}^{\text{cw}} + R_{\text{CO}_2}^{\text{pl}} + R_{\text{CO}_2}^{\text{cyt}}$ by Eq. 7.14a). Thus, our estimate based on the diffusion of CO_2 across each barrier is consistent with the measured values of mesophyll resistance.

There are many assumptions and specific choices for parameter values involved in the calculation of $R_{\text{CO}_2}^{\text{mes}}$ here. For instance, we let A^{mes}/A be 20, while many leaves have values from 30 to 40; the latter ratios would reduce our $R_{\text{CO}_2}^{\text{mes}}$ to 3 to 4 sec/cm . On the other hand, the cell walls of many mesophyll cells are $2 \mu\text{m}$ thick, which would increase our calculated $R_{\text{CO}_2}^{\text{cw}}$ to about 2 sec/cm . Permeability coefficients of the plasmalemmas of mesophyll cells for CO_2 have so far not been adequately measured. Our assumed value of 10^{-2} cm/sec is probably a lower limit. In particular, $K_{\text{CO}_2}^{\text{pl}}$ is most likely at least ten times higher than $K_{\text{H}_2\text{O}}^{\text{pl}}$, while the diffusion coefficients of both species are about the same (within a factor of two of each other). Thus, $P_{\text{CO}_2}^{\text{pl}}$ may be larger than 10^{-2} cm/sec , a reasonable value for $P_{\text{H}_2\text{O}}^{\text{pl}}$ ($P_j = D_j K_j / \Delta x$, Eq. 1.9, where Δx^{pl} is of course the same in each case). A higher value for $P_{\text{CO}_2}^{\text{pl}}$ would decrease our estimate for $R_{\text{CO}_2}^{\text{pl}}$, and thus for $R_{\text{CO}_2}^{\text{mes}}$.

Chloroplast resistance. The last two structural resistances encountered by the CO₂ used in photosynthesis are due to the chloroplasts ($R_{\text{CO}_2}^{\text{chl}} = R_{\text{CO}_2}^{\text{clm}} + R_{\text{CO}_2}^{\text{stroma}}$, Eq. 7.14b). As is the case for the plasmalemma, the resistance of the chloroplast limiting membranes ($R_{\text{CO}_2}^{\text{clm}}$) would be extremely large for the diffusion of HCO₃⁻ and moderate to low for the diffusion of CO₂. Numerous experiments indicate that the chloroplast limiting membranes are actually rather permeable to small solutes, so that $R_{\text{CO}_2}^{\text{clm}}$ may be lower than $R_{\text{CO}_2}^{\text{pl}}$, which we estimated to be at most 5 sec/cm. Since Δx^{stroma} might be 0.3 to 1 μm , while Δx^{cyt} is about 0.2 μm and the other pertinent parameters (A^{mes}/A , D_{CO_2} , K_{CO_2}) are approximately the same in the two cases, $R_{\text{CO}_2}^{\text{stroma}}$ could be a few times larger than $R_{\text{CO}_2}^{\text{cyt}}$, which we calculated to be only 0.1 sec/cm (if the pH in the chloroplast stroma were near or above 7, this would increase $K_{\text{CO}_2}^{\text{chl}}$ above unity and reduce $R_{\text{CO}_2}^{\text{stroma}}$ accordingly). In any case, $R_{\text{CO}_2}^{\text{stroma}}$ is a relatively small resistance.

At the present time, all we can really do is estimate an upper limit of 5 sec/cm for the resistance to the diffusion of CO₂ into chloroplasts and across their stroma. Measurement of $R_{\text{CO}_2}^{\text{chl}}$ *in vivo* is also rather difficult—analysis of available data indicates that most likely it is less than 4 sec/cm. Although active transport or facilitated diffusion of CO₂ or HCO₃⁻ into chloroplasts may occur and thus lower the effective resistance, the experimental values for $R_{\text{CO}_2}^{\text{chl}}$ are compatible with diffusion of CO₂ across the chloroplast limiting membranes.

Photosynthesis. The flux of CO₂ which gets into the chloroplasts represents the true (gross) rate of photosynthesis per unit leaf area, $J_{\text{CO}_2}^{\text{ps}}$. In the steady state, $J_{\text{CO}_2}^{\text{ps}}$ is the same as the flux entering the leaf, J_{CO_2} , corrected for any other reactions evolving or consuming carbon dioxide, as we will consider later. Most evidence indicates that CO₂, not HCO₃⁻, is the substrate for photosynthesis, and we will therefore focus our attention on CO₂. We will represent the rate of photosynthesis per unit *volume* of chloroplasts by v_{CO_2} , which can have units of ($\mu\text{moles of CO}_2 \text{ fixed/sec}$)/cm³. If chloroplasts have an average or effective thickness Δx^{chl} in a direction perpendicular to the plasmalemma, the rate of photosynthesis per unit leaf area ($J_{\text{CO}_2}^{\text{ps}}$) can be represented as

$$J_{\text{CO}_2}^{\text{ps}} = A^{\text{mes}} v_{\text{CO}_2} \Delta x^{\text{chl}} / A \quad (7.16)$$

where the factor A^{mes}/A is necessary in order to relate the chloroplast area to the leaf area. We can also derive Equation 7.16 by regarding the chloroplasts as a flat layer of average thickness (Δx^{chl}) which occupies some area, e.g., A^{mes} . The rate of photosynthesis in this volume ($v_{\text{CO}_2} A^{\text{mes}} \Delta x^{\text{chl}}$) is equal to the gross CO₂ flux per unit leaf area times the leaf area corresponding to A^{mes} , $J_{\text{CO}_2}^{\text{ps}} A$.

Observations indicate that the rates of CO_2 fixation by leaves and by isolated chloroplasts are proportional to the carbon dioxide level over the lower range of concentrations, and eventually reach an upper limit when the CO_2 concentration is raised sufficiently high. One way to describe such behavior is with a Michaelis-Menten type of expression:

$$v_{\text{CO}_2} = \frac{V_{\text{max}_{\text{CO}_2}} c_{\text{CO}_2}^{\text{chl}}}{K_{\text{M}_{\text{CO}_2}} + c_{\text{CO}_2}^{\text{chl}}} \quad (7.17)$$

where $V_{\text{max}_{\text{CO}_2}}$ is the maximum rate of CO_2 fixation per unit volume and $K_{\text{M}_{\text{CO}_2}}$ is essentially a Michaelis constant for CO_2 fixation, i.e., the value of $c_{\text{CO}_2}^{\text{chl}}$ at which v_{CO_2} equals $\frac{1}{2} V_{\text{max}_{\text{CO}_2}}$ —cf. Equation 3.27, $J_j^{\text{in}} = J_j^{\text{in}}_{\text{max}} c_j^0 / (K_j + c_j^0)$, and the discussion which follows it. Although convenient, using a Michaelis-Menten type of expression for the photosynthetic rate per unit volume may not always be justified for such a complicated series of reactions as photosynthesis (see Gaastra in Evans; Lommen *et al.*; Waggoner, and Zelitch).

In Equation 7.17 $V_{\text{max}_{\text{CO}_2}}$ and, to some extent, $K_{\text{M}_{\text{CO}_2}}$ depend on illumination level and temperature. For instance, $V_{\text{max}_{\text{CO}_2}}$ is zero in the dark, since photosynthesis then ceases, i.e., v_{CO_2} is zero in the dark. $V_{\text{max}_{\text{CO}_2}}$ is directly proportional to the illumination level up to about 1000 to 3000 lux, depending on the particular leaf. If we continually increase the illumination, $V_{\text{max}_{\text{CO}_2}}$ can reach an upper limit, its value for light saturation—this often occurs at 10,000 to 40,000 lux (photosynthesis in the nonphotorespiring C-4 plants, introduced below, is not light-saturated at even 100,000 lux). Photosynthesis is optimal at certain temperatures, generally from 30° to 40° . For such plants, $V_{\text{max}_{\text{CO}_2}}$ increases as the leaf temperature is raised to 35° or so, and then decreases as the temperature is increased even further (see Heath; Idso *et al.*, and Zelitch).

The values of $V_{\text{max}_{\text{CO}_2}}$ at light saturation and at the optimal temperatures for photosynthesis vary with the particular plant species, but they are generally from 2 to 10 $\mu\text{moles}/\text{cm}^3\text{-sec}$. We can also estimate $V_{\text{max}_{\text{CO}_2}}$ from measurements of the maximum rates of CO_2 fixation by isolated chloroplasts. These maximum rates—which are sustained for only rather short periods and are for optimal conditions—can be 300 μmoles of CO_2 fixed/mg chlorophyll-hr (the customary units), which is equal to approximately 3 $\mu\text{moles}/\text{cm}^3\text{-sec}$ (1 mg chlorophyll is contained in about 0.030 cm^3 of chloroplasts *in vivo*). The activity of ribulose-1,5-diphosphate carboxylase *in vitro* can approach rates equivalent to 500 $\mu\text{moles}/\text{mg}$ chlorophyll-hr. Usually, the estimates of $V_{\text{max}_{\text{CO}_2}}$ using isolated chloroplasts or enzymes are somewhat lower than its values determined for a leaf. Recent measurements using

isolated chloroplasts indicate that $K_{M_{CO_2}}$ is approximately 10 μ M, while studies with whole leaves suggest that it is closer to 5 μ M (see Zelitch).

In Chapter 5 we noted that the photon processing time for a photosynthetic unit of 250 chlorophylls could be 0.01 sec (p. 241). If 8 photons are required per CO₂, then 1 CO₂ can be fixed in 0.01 sec per 2000 chlorophylls, or $\frac{1}{20}$ CO₂/chlorophyll-sec. A chloroplast contains about 25 mM chlorophyll (p. 201) which is 25 μ moles chlorophyll/cm³. Hence, v_{CO_2} in this case would be (25 μ moles chlorophyll/cm³)($\frac{1}{20}$ CO₂/chlorophyll-sec), or 1.3 μ moles CO₂ fixed/cm³-sec. Since $c_{CO_2}^{chl}$ is generally limiting for photosynthesis under high illumination, this v_{CO_2} may be only $\frac{1}{2}$ to $\frac{1}{5}$ $V_{max_{CO_2}}$ at that temperature. Conversely, the 0.01 sec length of the processing time may be due mainly to suboptimal CO₂ levels in the chloroplasts. A related factor is the increase during this century in atmospheric CO₂ from about 300 to 320 ppm by volume because of the extensive burning of fossil fuels. Other things being equal, this increase in $c_{CO_2}^{ta}$ will lead to a rise in $c_{CO_2}^{chl}$ and hence in v_{CO_2} (cf. Eq. 7.17).

Respiration and photorespiration. So far, the only process involving CO₂ we have considered in this chapter is photosynthesis. However, we cannot neglect the CO₂ produced within mesophyll cells by respiration and photorespiration. If mitochondrial respiration in leaf cells were the same in the light as in the dark, when it can be readily measured, respiration would produce about 5% as much CO₂ as is consumed by photosynthesis at a moderate illumination. In those plants which photorespire, the rate of light-stimulated production of CO₂ by photorespiration is often about 30% of the rate of CO₂ fixation into photosynthetic products.

Since we have already considered respiration in Chapter 6, we will next briefly comment on photorespiration (see Hatch *et al.*, Tolbert, and Zelitch). The evolution of CO₂ in photorespiration apparently occurs in the leaf cell organelles known as peroxisomes, and the carbon atom of CO₂ comes from the carboxyl group of glycolate (CH₂OHCOOH), the immediate precursor being glyoxylate (CHOCOOH) and/or glycine (NH₂CH₂COOH). Glycolate is formed in the chloroplasts as a product of photosynthesis. Since photorespiration depends on substrates produced during photosynthesis, it is influenced by illumination level and by temperature, although not quite in the same manner as photosynthesis. For instance, photosynthesis usually doubles in going from 20° to 30°, while photorespiration often triples over this interval of leaf temperature. As far as CO₂ fixation is concerned, photorespiration apparently undoes what photosynthesis has done. We might then ask how photorespiration benefits a plant—a question that has no entirely convincing answer as yet.

A number of plants do not photorespire. Most of these nonphotorespiring plants are tropical monocots, and all tend to have a leaf anatomy different from that of plants with high rates of photorespiration. Nonphotorespirers have a conspicuous group of chloroplast-containing cells surrounding the vascular bundles known as the *bundle sheaths* (the bundle sheaths in photorespirers tend to have smaller cells with few or no chloroplasts). Outside a bundle sheath in nonphotorespirers are mesophyll cells whose chloroplasts fix CO_2 into carbohydrates by means of a C-4 dicarboxylic acid pathway elaborated by M. D. Hatch and C. R. Slack in the 1960s (see Hatch and Slack). In this pathway, CO_2 is incorporated into phosphoenolpyruvate yielding oxaloacetate, which is then rapidly converted to malate and aspartate. For all chloroplasts in photorespiring plants and the chloroplasts of the bundle sheath cells of nonphotorespirers, photosynthesis uses the ordinary C-3 pathway of the Calvin cycle—known since the 1940s—where CO_2 is incorporated into ribulose-1,5-diphosphate yielding two molecules of 3-phosphoglycerate. Nonphotorespiring plants in arid regions are often exposed to water stress, and tend to have high values of $R_{\text{wv}}^{\text{leaf}}$ and $R_{\text{CO}_2}^{\text{leaf}}$. In such cases, it is an obvious advantage for such plants not to release any CO_2 by photorespiration.

Comprehensive CO_2 resistance network. We can now develop an analytical framework in which to represent CO_2 fixation in photosynthesis and its evolution in respiration and photorespiration. The net flux of CO_2 into a leaf (J_{CO_2}) is a measure of the apparent (net) CO_2 assimilation rate by photosynthesis. The gross or “true” rate of photosynthesis ($J_{\text{CO}_2}^{\text{ps}}$) minus the rate of CO_2 evolution by respiration and photorespiration per unit leaf area ($J_{\text{CO}_2}^{\text{r+pr}}$) is related to J_{CO_2} as follows:

$$J_{\text{CO}_2} = J_{\text{CO}_2}^{\text{ps}} - J_{\text{CO}_2}^{\text{r+pr}} \quad (7.18)$$

Equation 7.18 summarizes the overall steady state balance of CO_2 fluxes for leaves.

We will find it convenient to consider CO_2 fluxes and resistances for photosynthesis, respiration, and photorespiration using an electrical circuit (Fig. 7.5). The sources of CO_2 for photosynthesis are the turbulent air surrounding a leaf (represented by the E battery in Fig. 7.5) and respiration plus photorespiration (the e battery). The E battery actually corresponds to the drop in CO_2 concentration from the turbulent air surrounding a leaf to the enzymes of photosynthesis, $c_{\text{CO}_2}^{\text{ta}} - c_{\text{CO}_2}^{\text{chl}}$, which represents a driving force on carbon dioxide. The batteries lead to currents which correspond to fluxes of CO_2 , e.g., I , the current from the E battery, corresponds to J_{CO_2} , while i represents the flux of CO_2 emanating from respiration and photo-

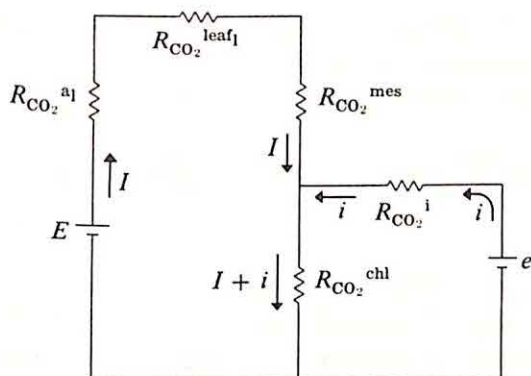


Figure 7.5

Electrical circuit indicating the resistances affecting photosynthesis, respiration, and photorespiration. The sources of CO₂ are the turbulent air surrounding the leaf (represented by the battery of electromotive force E) and respiration plus photorespiration (the e battery). The current I corresponds to the net CO₂ influx into the leaf (J_{CO_2}), i represents CO₂ evolution by respiration and photorespiration ($J_{\text{CO}_2}^{\text{r+pr}}$), and $I + i$ corresponds to gross photosynthesis ($J_{\text{CO}_2}^{\text{ps}}$).

respiration, $J_{\text{CO}_2}^{\text{r+pr}}$. The current I crosses the resistances $R_{\text{CO}_2}^{\text{ai}}$, $R_{\text{CO}_2}^{\text{leaf1}}$, and $R_{\text{CO}_2}^{\text{mes}}$ before being joined by i (see Fig. 7.5). The current i encounters $R_{\text{CO}_2}^{\text{i}}$, which is the resistance to the movement of carbon dioxide out of mitochondria and peroxisomes and then across a short distance in the cytoplasm. Both I and i cross the resistance $R_{\text{CO}_2}^{\text{chl}}$, since CO₂ coming from the surrounding air as well as that evolved by respiration and photorespiration can be used for photosynthesis in the chloroplasts (this is a way of paraphrasing Eq. 7.18).

To analyze the electrical circuit in Figure 7.5, we will make use of Ohm's law ($\Delta E = IR$) and Kirchhoff's second law. The latter rule—which is also known as the *loop theorem* and is a consequence of the conservation of energy—states that the overall change in electrical potential in going completely around a closed loop is zero. By considering a complete pathway around the left part of the electrical circuit in Figure 7.5, we obtain the following relationship:

$$E - I(R_{\text{CO}_2}^{\text{ai}} + R_{\text{CO}_2}^{\text{leaf1}} + R_{\text{CO}_2}^{\text{mes}}) - (I + i)(R_{\text{CO}_2}^{\text{chl}}) = 0 \quad (7.19)$$

When the circuit is viewed from the E battery, which represents the source of CO_2 in the turbulent air surrounding the leaf, it has an effective resistance of E/I . We can rearrange Equation 7.19 to give us this value of E/I :

$$\frac{E}{I} = R_{\text{CO}_2}^{\text{total}} = R_{\text{CO}_2}^{\text{ai}} + R_{\text{CO}_2}^{\text{leafi}} + R_{\text{CO}_2}^{\text{mes}} + \left(\frac{I+i}{I}\right)(R_{\text{CO}_2}^{\text{chl}}) \quad (7.20)$$

where $R_{\text{CO}_2}^{\text{total}}$ is the total effective resistance of the electrical circuit.

Equation 7.20 indicates that $R_{\text{CO}_2}^{\text{total}}$ depends on the current i . Such dependency of an *effective* resistance on current is a common occurrence for a complicated electrical circuit. Moreover, it has an obvious analogy in CO_2 fluxes. Since $R_{\text{CO}_2}^{\text{total}}$ increases as i increases, I must then decrease when E is constant (Eq. 7.20). Likewise, if respiration or photorespiration increase (current i), the net photosynthetic rate of the leaf (current I) would be expected to decrease when other factors are unchanged.

Compensation points. The atmospheric CO_2 concentration at which the CO_2 evolved by respiration and photorespiration is exactly compensated for by a CO_2 consumption in photosynthesis is known as the CO_2 *compensation point*. We can use Figure 7.5 to demonstrate the CO_2 compensation point for photosynthesis in terms of forces and fluxes. If we continuously decrease E (which corresponds to $c_{\text{CO}_2}^{\text{ta}} - c_{\text{CO}_2}^{\text{chl}}$), the current I (i.e., J_{CO_2}) will decrease and eventually become zero when E is equal to $i R_{\text{CO}_2}^{\text{chl}}$. Hence, if we decrease the concentration of CO_2 in the turbulent air surrounding an illuminated leaf, the net fixation of carbon dioxide eventually ceases and we have reached the CO_2 compensation point.

In a photorespiring plant the CO_2 compensation point is considerably higher than in a plant which does not photorespire. By our electrical circuit analogy, E equals $i R_{\text{CO}_2}^{\text{chl}}$ at the CO_2 compensation point, and i (which corresponds to $J_{\text{CO}_2}^{\text{r+pr}}$) is of course larger when photorespiration is appreciable. Most nonphotorespiring plants, e.g., sugarcane, sorghum, maize, some species of saltbush (e.g., *Atriplex rosea*), bermuda grass, Sudan grass, *Amaranthus*, and many other tropical and subtropical species, have CO_2 compensation points of 5-10 ppm CO_2 (by volume) in the turbulent air. Most dicotyledons and temperate monocots photorespire, e.g., wheat, tobacco, *Pelargonium*, orchard grass, and tomato, and have CO_2 compensation points of 40-100 ppm CO_2 at 25° . A few species, e.g., soybean and *Atriplex hastata*, have intermediate CO_2 compensation points near 35 ppm CO_2 . The CO_2 compensation points generally increase with increasing temperature and decreasing illumination, the values given being appropriate at 25° when light is not limiting for photosynthesis (see Zelitch).

If we continuously reduce the amount of light incident on a leaf from the value for direct sunlight, we eventually reach an illumination where there is no net photosynthesis. This illumination, where J_{CO_2} is zero, is known as the *light compensation point* for photosynthesis. Since photorespiration depends on photosynthetic products, both photorespiration and gross photosynthesis decrease as the illumination is lowered. Hence, the light compensation point for leaves is approximately the same for photorespirers and nonphotorespirers—at 20° and 300 ppm CO₂ it is usually about 300–500 lux for photorespirers, and 200–400 lux for nonphotorespirers (see Heath and Zelitch). The light compensation point is quite important in our consideration of the leaf canopy in the next chapter. For example, the uppermost leaves reach the light compensation point on dark days or at sunset, while leaves shaded by many overlying leaves can actually be at (or below) the light compensation point when the exposed leaves have appreciable net rates of photosynthesis.

At either compensation point, I is zero when E equals $i R_{\text{CO}_2}^{\text{chl}}$ (cf. Fig. 7.5). Likewise, J_{CO_2} is zero when $c_{\text{CO}_2}^{\text{ta}} - c_{\text{CO}_2}^{\text{chl}}$ equals $J_{\text{CO}_2}^{\text{r+pr}} R_{\text{CO}_2}^{\text{chl}}$. For the CO₂ compensation point, we reduced $c_{\text{CO}_2}^{\text{ta}}$ until J_{CO_2} became zero. For the light compensation point, $c_{\text{CO}_2}^{\text{ta}}$ is unchanged; instead, $c_{\text{CO}_2}^{\text{chl}}$ increases when the illumination decreases with the accompanying decrease in carbon dioxide fixation by photosynthesis. If we lower the illumination below the light compensation point, J_{CO_2} reverses its direction, which means a net flux of CO₂ out of the leaf (as happens at night). At night, I becomes equal to $-i$, hence the current across $R_{\text{CO}_2}^{\text{chl}}$ is zero, i.e., gross photosynthesis ceases (cf. Fig. 7.5). The respiratory flow of carbon dioxide out of the leaf is then driven by the higher CO₂ concentration in the mitochondria than in the turbulent air, and encounters the resistances $R_{\text{CO}_2}^{\text{i}}$, $R_{\text{CO}_2}^{\text{mes}}$, $R_{\text{CO}_2}^{\text{leafi}}$, and $R_{\text{CO}_2}^{\text{ai}}$, in that order. Thus, our electrical circuit in Figure 7.5 is able to portray the CO₂ compensation point, the light compensation point, as well as the general interrelations of the fluxes of CO₂ for photosynthesis, photorespiration, and respiration in the light and dark.

Fluxes of CO₂. Using Equation 7.20, which we derived for the electrical circuit in Figure 7.5, we can relate the apparent rate of photosynthesis (J_{CO_2}) to $R_{\text{CO}_2}^{\text{total}}$ and $\Delta c_{\text{CO}_2}^{\text{total}}$. We have already noted that E corresponds to $c_{\text{CO}_2}^{\text{ta}} - c_{\text{CO}_2}^{\text{chl}}$, which here is $\Delta c_{\text{CO}_2}^{\text{total}}$. The current $I + i$ represents $J_{\text{CO}_2}^{\text{ps}}$, while I corresponds to J_{CO_2} , which is $J_{\text{CO}_2}^{\text{ps}} - J_{\text{CO}_2}^{\text{r+pr}}$ by Equation 7.18. Hence, the factor $(I + i)/I$ in Equation 7.20 represents $J_{\text{CO}_2}^{\text{ps}}/(J_{\text{CO}_2}^{\text{ps}} - J_{\text{CO}_2}^{\text{r+pr}})$, which equals $1/(1 - J_{\text{CO}_2}^{\text{r+pr}}/J_{\text{CO}_2}^{\text{ps}})$. Upon substituting these corresponding forms for E and $(I + i)/I$ into Equation 7.20, we obtain the following comprehensive expression for the net (apparent) rate of photo-

synthesis per unit leaf area:

$$J_{\text{CO}_2} = \frac{\Delta c_{\text{CO}_2}^{\text{total}}}{R_{\text{CO}_2}^{\text{total}}} = \frac{c_{\text{CO}_2}^{\text{ta}} - c_{\text{CO}_2}^{\text{chl}}}{R_{\text{CO}_2}^{\text{ai}} + R_{\text{CO}_2}^{\text{leaf}_1} + R_{\text{CO}_2}^{\text{mes}} + \frac{R_{\text{CO}_2}^{\text{chl}}}{1 - \frac{J_{\text{CO}_2}^{\text{r+pr}}}{J_{\text{CO}_2}^{\text{ps}}}}} \quad (7.21)$$

Next, let us calculate J_{CO_2} using Equation 7.21. We will let $R_{\text{CO}_2}^{\text{ai}}$ be 0.8 sec/cm, $R_{\text{CO}_2}^{\text{leaf}_1}$ be 3.5 sec/cm, $R_{\text{CO}_2}^{\text{mes}}$ be 3.5 sec/cm, $R_{\text{CO}_2}^{\text{chl}}$ be 2.0 sec/cm, and $J_{\text{CO}_2}^{\text{r+pr}}/J_{\text{CO}_2}^{\text{ps}}$ be 0.3. Using Equation 7.21, the value of the total resistance for the entry of CO_2 , $R_{\text{CO}_2}^{\text{total}}$, is $0.8 + 3.5 + 3.5 + (2.0)/(1.0 - 0.3)$, or 10.7 sec/cm. The usual concentration of 320 ppm of CO_2 in the turbulent air corresponds to $(320)(0.0410)$, or 13 nmoles of CO_2/cm^3 at 20° and a pressure of 1 bar (conversion factor in Table 7.2). Although we do not have reliable measurements of $c_{\text{CO}_2}^{\text{chl}}$, it may be about 3 nmoles/ cm^3 for a photorespiring plant under moderately high illuminations. Again employing Equation 7.21, we calculate that J_{CO_2} equals $(13 - 3 \text{ nmoles}/\text{cm}^3)/(10.7 \text{ sec}/\text{cm})$, or 0.9 nmole of $\text{CO}_2/\text{cm}^2\text{-sec}$.

So far, we have considered CO_2 diffusing into the leaf only across the lower epidermis. In the general case, CO_2 can move in across the upper epidermis as well, which we can incorporate into our considerations by appropriately reducing the resistance $R_{\text{CO}_2}^{\text{ai}} + R_{\text{CO}_2}^{\text{leaf}_1}$ ($R_{\text{CO}_2}^{\text{mes}}$ and $R_{\text{CO}_2}^{\text{chl}}$ are unaffected when CO_2 diffuses in both sides of the leaf). When 30% of the CO_2 diffuses in through the upper side of the leaf, the effective resistance between the turbulent air and the surfaces of the mesophyll cells would be only 70% as great as $R_{\text{CO}_2}^{\text{ai}} + R_{\text{CO}_2}^{\text{leaf}_1}$.^{*} For instance, $R_{\text{CO}_2}^{\text{ai}} + R_{\text{CO}_2}^{\text{leaf}_1}$ here is 4.3 sec/cm, so that the effective resistance of this part of the pathway is reduced to $(0.70)(4.3)$, or 3.0 sec/cm, if 30% of the CO_2 enters through the upper epidermis. $R_{\text{CO}_2}^{\text{total}}$ is then 9.4 sec/cm. The relatively small decrease in $R_{\text{CO}_2}^{\text{total}}$ from 10.7 sec/cm to 9.4 sec/cm reflects in this case the substantial resistance of the unaffected nongaseous parts of the pathway for CO_2 movement ($R_{\text{CO}_2}^{\text{mes}}$ and $R_{\text{CO}_2}^{\text{chl}}$) compared with the gaseous parts ($R_{\text{CO}_2}^{\text{ai}}$ and $R_{\text{CO}_2}^{\text{leaf}_1}$). For an $R_{\text{CO}_2}^{\text{total}}$ of 9.4 sec/cm, J_{CO_2} here is $(10/9.4)$, or 1.1 nmoles/ $\text{cm}^2\text{-sec}$, by Equation 7.21.

As we would expect, the value of J_{CO_2} varies considerably with plant species, temperature, and illumination (see Šetlik and Zelitch). For instance, J_{CO_2} is often 0.5 to 0.7 nmole/ $\text{cm}^2\text{-sec}$ for leaves of trees and many other woody species under daytime illumination, indicating an $R_{\text{CO}_2}^{\text{total}}$ near

^{*} See p. 323 for our discussion of the analogous situation in transpiration.

20 sec/cm. The lower $R_{\text{CO}_2}^{\text{total}}$, the greater J_{CO_2} by Equation 7.21, which means the higher would be the net rate of photosynthesis. Certain crop plants, e.g., sugar beet, soybean, and tobacco, have a total resistance for carbon dioxide close to 6 sec/cm. For moderately high illumination levels and leaf temperatures near 30°, J_{CO_2} would then be about 2 nmoles/cm²-sec.

When photorespiration is negligible, J_{CO_2} tends to be larger. First, $J_{\text{CO}_2}^{\text{r+pr}}$ is less for nonphotorespiring plants, in effect reducing $R_{\text{CO}_2}^{\text{total}}$ (cf. Eqs. 7.20 and 7.21). Second, $c_{\text{CO}_2}^{\text{chl}}$ tends to be lower for nonphotorespirers, which increases $\Delta c_{\text{CO}_2}^{\text{total}}$ (Eq. 7.21). (V_{maxCO_2} for nonphotorespiring plants usually increases as the illumination is raised all the way to the value of full sunlight—about 100,000 lux—which counteracts the effect of lower $c_{\text{CO}_2}^{\text{chl}}$ on v_{CO_2} , cf. Eq. 7.17.) Finally, some nonphotorespiring plants have a high value for A^{mes}/A , e.g., above 50. Such a large surface area of the mesophyll cells tends to lower $R_{\text{CO}_2}^{\text{mes}}$ and $R_{\text{CO}_2}^{\text{chl}}$, which are inversely proportional to A^{mes}/A (cf. Eq. 7.15). Under optimal conditions of high illumination and a leaf temperature of 35°, $R_{\text{CO}_2}^{\text{total}}$ can be as low as 3 sec/cm for nonphotorespiring plants. Thus, for bermuda grass, maize, sugarcane, and some other tropical grasses, as well as for a few dicots that do not photorespire, J_{CO_2} can be as high as 4 nmoles/cm²-sec.

The important influence of A^{mes}/A on J_{CO_2} deserves special emphasis. A^{mes}/A can be two or more times larger for sun leaves than for shade leaves on the same plant; this reduces $R_{\text{CO}_2}^{\text{total}}$ and thus enhances the photosynthetic rates of sun leaves compared with shade leaves. The large area of the cell walls of mesophyll cells which is available for CO₂ diffusion keeps $R_{\text{CO}_2}^{\text{cw}}$ and the other CO₂ resistances in the mesophyll cells at reasonable values. For instance, if the mesophyll cells were tightly packed into a layer with no intervening air spaces, A^{mes}/A could equal unity. Instead of the value 1 sec/cm that we calculated above for the cell wall resistance using an A^{mes}/A of 20, $R_{\text{CO}_2}^{\text{cw}}$ alone would then be 20 sec/cm. Thus, the evolution of a leaf anatomy permitting a large value for A^{mes}/A allows $R_{\text{CO}_2}^{\text{mes}}$ and $R_{\text{CO}_2}^{\text{chl}}$ to be reasonably low.

Many units are used for expressing photosynthesis and CO₂ fluxes for leaves. To facilitate conversions, factors for some of the more common units are summarized in Table 7.2. For example, 17 mg CO₂/dm²-hr corresponds to (17)(0.0631), or 1.1 nmoles/cm²-sec. The amount of chlorophyll per unit leaf area generally ranges from 0.01 to 0.08 mg chlorophyll/cm² of leaf, with a value of 0.04 being representative. Thus, 1.1 nmoles of CO₂ fixed/cm²-sec might correspond to (1.1 nmoles/cm²-sec)/(0.04 mg chlorophyll/cm²), or 27 nmoles/mg chlorophyll-sec, which equals 100 μ moles of CO₂ fixed/mg chlorophyll-hr.

Relationships between J_{wv} and J_{CO_2} . From the values of J_{wv} and J_{CO_2} we can calculate the ratio of water lost by a leaf to carbon dioxide fixed. Since J_{wv} may be $0.41 \mu\text{mole}/\text{cm}^2\text{-sec}$ for a typical mesophyte (p. 323), and J_{CO_2} for such plants is often about $1.1 \text{ nmoles}/\text{cm}^2\text{-sec}$, approximately $(410 \text{ nmoles H}_2\text{O}/\text{cm}^2\text{-sec})/(1.1 \text{ nmoles CO}_2/\text{cm}^2\text{-sec})$, or 370 H_2O 's may be lost by a leaf for every CO_2 fixed. This substantial water loss per CO_2 fixed is not a problem when plenty of water is available for transpiration. Plants in such environments often have a low value for R_{wv}^{total} , which goes with a slightly reduced value for $R_{CO_2}^{\text{total}}$ and hence somewhat higher rates of photosynthesis. Certain nonphotorespiring crops have low values for $R_{CO_2}^{\text{total}}$, near $4 \text{ sec}/\text{cm}$, and at moderate illuminations exposed leaves can fix one CO_2 for every 100 waters transpired (J_{wv} depends of course on c_{wv}^m and c_{wv}^{ta}). (See Levitt.)

Any loss of water can be potentially harmful for plants growing in arid regions, many of which have evolved a novel way of fixing CO_2 without a large value for J_{wv}/J_{CO_2} . For example, many species in the family Crassulaceae and other desert succulents have their stomates closed during the day. This greatly reduces transpiration, but also essentially eliminates the net influx of CO_2 during the day. The stomates open during the night when the leaf temperature is much lower than daytime values. The concentration of water vapor in the pores of the cell walls of mesophyll cells (c_{wv}^m) is then much lower, markedly reducing the rate of transpiration. At night, CO_2 diffuses in through the open stomates and is fixed into malate (e.g., by carboxylation of phosphoenolpyruvate) and other organic acids. During the next day light causes the production of ATP and NADPH_2 in the chloroplasts. These compounds are then used for the reduction of the organic acids to carbohydrates, which are often stored in the form of starch. Plants which accumulate malate, citrate, and other organic acids during the night and then reduce these compounds to the level of carbohydrates during the day are referred to as CAM (Crassulacean acid metabolism) plants, since such reactions were first observed in the Crassulaceae. Most CAM plants tend to be slow-growing. However, because the stomates are not open during the day, water is conserved by such plants, so that J_{wv}/J_{CO_2} averaged over 24 hours is quite low, e.g., only about 30 molecules of H_2O may be lost per CO_2 fixed into carbohydrates (see Zelitch).

Changes in the resistances of the unstirred air layers bounding a leaf and of the stomates have a greater influence on the flux of water vapor than on that of carbon dioxide. Specifically, the total resistance for water vapor diffusion (R_{wv}^{total}) can equal $R_{wv}^{\text{ai}} + R_{wv}^{\text{leaf}}$, while $R_{CO_2}^{\text{ai}} + R_{CO_2}^{\text{leaf}}$ is

generally less than half of the total resistance for carbon dioxide diffusion ($R_{\text{CO}_2}^{\text{total}}$). Thus, changes in wind speed, which influence the thickness of the unstirred layers and hence R_j^a , have a smaller fractional effect on $R_{\text{CO}_2}^{\text{total}}$ than on R_{wv}^{total} . Similarly, R_{wv}^{total} can be reduced appreciably by partial stomatal closure, which leads to a smaller fractional reduction in $R_{\text{CO}_2}^{\text{total}}$. In most plants the stomates begin to close when the illumination decreases below 10,000 lux. The accompanying increase in stomatal resistance decreases net photosynthesis but decreases transpiration even more, and so water is not unnecessarily lost from the plant.

Energy fluxes

So far in this chapter we have been concerned with water and carbon dioxide fluxes into and out of leaves. The underlying principle is that gas molecules will spontaneously diffuse toward regions of lower concentration. We have also noted that the surrounding air can have a temperature different from the leaves. This will lead to a spontaneous flux of heat toward the region with the lower temperature. Such heat movement is only one of the ways in which energy can enter or leave a leaf.

The principle of the conservation of energy (the first law of thermodynamics) states that energy cannot be created or destroyed, but only changed from one form to another. We will apply this principle to the energy balance of a leaf, which exists in an environment with many energy fluxes. We can summarize the various contributors to the energy balance of a leaf as follows:

absorbed solar radiation, absorbed infrared radiation from surroundings	emitted infrared radiation, heat convection, heat conduction, heat loss accompanying water evaporation	photosynthesis, other metabolism, leaf temperature changes
energy into leaf	— energy out of leaf	= energy storage by leaf
(7.22)		

Since heat moves *into* a leaf when its temperature is less than T^{ta} , Equation 7.22 actually describes the case where T^{leaf} is greater than the temperature of the surroundings. Also, when water condenses onto a leaf, the leaf will gain heat. In such cases, we can consider that the appropriate energy terms in Equation 7.22 simply change sign.

The various terms in Equation 7.22 differ greatly in magnitude. For instance, the energy storage terms generally are relatively small. As a basis for comparison, we will consider the average amount of solar radiation incident on the earth's atmosphere. This radiant flux, the solar constant, is about $2.00 \text{ cal/cm}^2\text{-min}$ (p. 171). The solar radiation absorbed by an exposed leaf is often about half of this number during the daytime. Hence, any process under $0.01 \text{ cal/cm}^2\text{-min}$ would correspond to less than 1% of the absorbed solar radiation for full sunlight. What is the amount of energy stored by photosynthesis? A typical net rate of CO_2 fixation by a photosynthetically active leaf is $1.1 \text{ nmoles/cm}^2\text{-sec}$ (p. 340), which is $66 \text{ nmoles/cm}^2\text{-min}$. As indicated in Chapter 5 (p. 216), about 114 kcal of energy are stored per mole of CO_2 fixed into photosynthetic products. Hence, photosynthesis by leaves might store $(66 \times 10^{-9} \text{ mole/cm}^2\text{-min})(114,000 \text{ cal/mole})$, or $0.0075 \text{ cal/cm}^2\text{-min}$, which is only about 1% of the rate of absorption of solar radiation under the same conditions. In some cases the rate of photosynthesis can be higher (cf. p. 341). However, we can generally ignore photosynthesis insofar as its contribution to the energy balance of a leaf is concerned. Other metabolic processes in a leaf, such as respiration and photorespiration, are usually less important on an energy basis than photosynthesis, so that they, too, can generally be ignored.

Let us now consider the amount of energy that can be stored because of changes of leaf temperature. For purposes of calculation, we will assume that a leaf has the high specific heat of water ($1.00 \text{ cal/g}^\circ\text{K}$ at 20°), is $400 \mu\text{m}$ (0.04 cm) thick, and has an overall density of 0.5 g/cm^3 —a leaf is often 50% air by volume. Hence, the mass per unit leaf area in this case is $(0.04 \text{ cm})(0.5 \text{ g/cm}^3)$, or 0.02 g/cm^2 . If $0.01 \text{ cal/cm}^2\text{-min}$ were stored by temperature changes in such a leaf, its temperature would rise at the rate of $(0.01 \text{ cal/cm}^2\text{-min})/[(1.00 \text{ cal/g}^\circ\text{K})(0.02 \text{ g/cm}^2)]$, or $0.5^\circ/\text{min}$. Since this is a faster temperature change than that sustained for very long periods by leaves, we can assume that very little energy is stored (or released) in the form of leaf temperature changes. Hence, all three energy storage terms indicated in Equation 7.22 usually are relatively small for a leaf.

When the energy storage terms in Equation 7.22 are ignored, the remaining contributors to the energy balance of a leaf are either radiation or heat

terms. We can then simplify our energy balance relation for a leaf as follows:

$$\begin{array}{ccc}
 \begin{array}{l} \text{absorbed solar radiation,} \\ \text{absorbed infrared radiation} \\ \text{from surroundings} \end{array} & & \begin{array}{l} \text{emitted infrared radiation,} \\ \text{heat convection,} \\ \text{heat conduction,} \\ \text{heat loss accompanying} \\ \text{water evaporation} \end{array} \\
 \underbrace{\hspace{1.5cm}} & & \underbrace{\hspace{1.5cm}} \\
 \text{energy into leaf} & \cong & \text{energy out of leaf}
 \end{array} \tag{7.23}$$

The heat conducted and convected from leaves is sometimes referred to as “sensible” heat, while that associated with the evaporation or the condensation of water is known as “latent” heat. In this section we will consider each of the terms in Equation 7.23. The unit we are using for energy flux, cal/cm²-min, is only one of several commonly employed. For instance, time could be expressed in seconds, and heat in ergs or joules. (See conversion factors in Table 7.2; also, note that 1.00 cal/cm²-min equals 0.697×10^6 ergs/cm²-sec, 697 watts/m²—a watt is a joule/sec—and 69.7 mwatts/cm².)

Solar radiation. Solar radiation can be incident on a leaf in many different ways, the most obvious one being *direct* sunlight. Also, sunlight can be *scattered* by molecules and particles in the atmosphere before striking the leaf. Finally, both the direct and the scattered solar radiation can be *reflected* by the surroundings toward the leaf. In Figure 7.6 we have summarized these various possibilities, which themselves lead to six different ways (all containing the letter *S* in the figure) by which solar radiation can impinge on a leaf. The individual energy fluxes may involve the upper surface of a leaf, its lower surface, or perhaps both surfaces. In Figure 7.6 the direct solar radiation (S^{direct}) is incident only on the upper surface of the leaf. In order to proceed with the analysis in a reasonable fashion, we will obviously need to make many simplifying assumptions and approximations (see Idso *et al.*; Robinson, and van Wijk).

Some of the solar radiation can be scattered or reflected from clouds before being incident on a leaf.* On a cloudy day, the diffuse sunlight emanating from the clouds (S^{cloud} or “cloudlight”) is substantial, while S^{direct} may be greatly reduced. For instance, a sky overcast by clouds 100 m thick might absorb or reflect away from the earth about 50% of the incident solar

* Generally, the term *scattering* denotes the irregular changes in the direction of light caused by small particles or molecules; *reflection* refers to the change in direction of radiation at a surface.

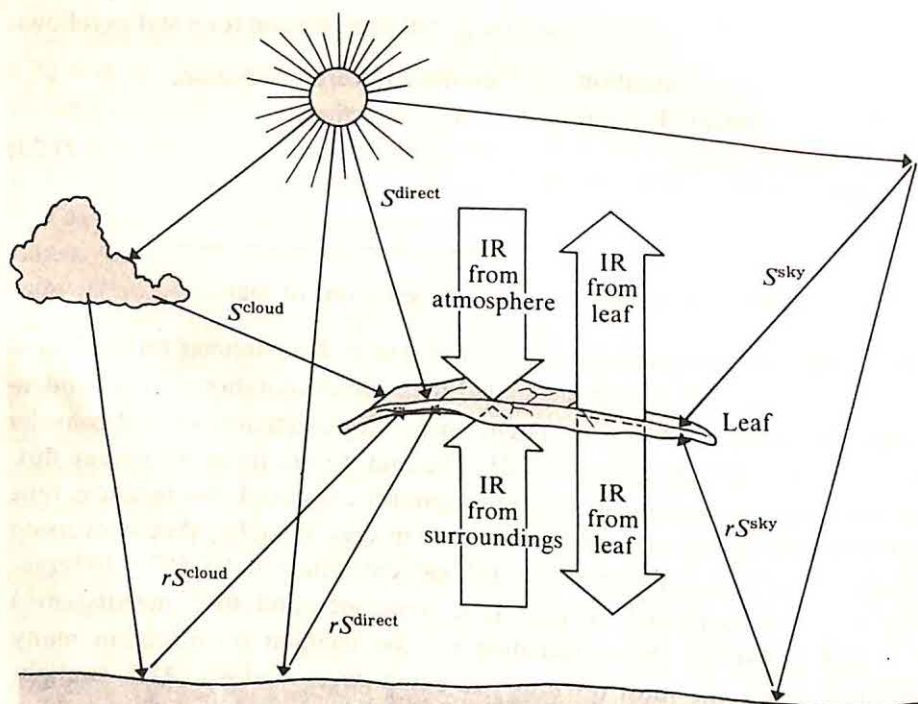


Figure 7.6

Schematic illustration of eight forms of radiant energy incident on an exposed leaf, and the infrared radiation emitted from its two surfaces.

radiation and diffusely scatter most of the rest toward the earth. A cloud 1 km thick will usually scatter somewhat less than 10% of the S_{direct} incident on it toward the earth (the actual numbers depend on the type and density of the clouds). Because the relative amounts of absorption, scattering, reflection, and transmission by clouds depend on wavelength, cloudlight has a somewhat different wavelength distribution than does direct solar radiation.

Solar radiation scattered by air molecules leads to S_{sky} , or "skylight." Skylight differs considerably from the wavelength distribution for S_{direct} , since S_{sky} is enriched in the shorter wavelengths (see Fig. 7.7). Rayleigh scattering of solar radiation by the molecules in the atmosphere depends essentially on $1/\lambda^4$ —light scattering from air-borne dust particles is approximately proportional to $1/\lambda^2$ —hence the shorter wavelengths are scattered more. Because of the Rayleigh scattering, a preponderance of the shorter wavelengths, e.g., below 500 nm, are actually scattered out of the direct solar

beam (cf. Fig. 7.7) and become skylight. This is the explanation for the blue color of the sky (and its skylight) during the daytime. In terms of energy, skylight (S^{sky}) can be up to 10% of S^{direct} on a cloudless day (Fig. 7.7).

For convenience, we will refer to the direct sunlight plus the cloudlight and skylight as the *global radiation*, S . The value of the global radiation varies with the time of day, the time of year, latitude, altitude, and with atmospheric conditions. Consequently, it is an important parameter to measure when considering the energy balance of a leaf. The maximum value of S is essentially $2.00 \text{ cal/cm}^2\text{-min}$, the solar radiation incident on the earth's atmosphere (p. 171). Because of scattering and absorption of solar radiation by atmospheric gases (Fig. 4.2), S on a cloudless day with the sun directly overhead in a dust-free sky is about $1.5 \text{ cal/cm}^2\text{-min}$ at 2000 m and $1.2 \text{ cal/cm}^2\text{-min}$ at sea level.

Sunlight may impinge on a leaf as direct solar radiation, cloudlight, or skylight. These three aspects of global radiation may first be reflected from the surroundings before being incident on a leaf (see Fig. 7.6). Although the reflected global radiation can be incident on the leaf from all angles, for a horizontal exposed leaf it falls primarily on the lower surface (Fig. 7.6). The reflected direct sunlight, cloudlight, and skylight often amount to 10 to 20% of the global radiation.

Each of these six forms of solar radiation can have a different wavelength distribution. Since the absorptivity depends on wavelength, the fraction of each one absorbed can in principle be different. Moreover, the fraction reflected also depends on wavelength, so that we could use a different reflectivity for S^{direct} , S^{cloud} , and S^{sky} . For simplicity, we will assume that the same absorptivity a applies to direct solar radiation, cloudlight, and skylight, as well as to the reflected forms of these radiations. We will also assume that the same reflectivity r applies to each component of the global radiation. We can then represent the absorption of all forms of solar radiation by a leaf as follows:

$$\begin{aligned} & \text{absorbed direct, scattered, and reflected solar radiation per unit leaf area} \\ & \cong a(S^{\text{direct}} + S^{\text{cloud}} + S^{\text{sky}}) + ar(S^{\text{direct}} + S^{\text{cloud}} + S^{\text{sky}}) \\ & = a(1 + r)S \end{aligned} \quad (7.24)$$

where the absorptivity (a) equals the fraction of the various forms of global radiant energy (S) absorbed by the leaf, and the reflectivity (r) is the fraction of the energy of the incoming sunlight, cloudlight, and skylight reflected onto the leaf.

Absorbed infrared radiation. In addition to the absorption of various forms of solar radiation, infrared or thermal radiation is also absorbed by

a leaf (cf. Eq. 7.23). Any object with a temperature above 0°K emits such "thermal" radiation, including the leaf surroundings as well as the sky. The peak in the spectral distribution of thermal radiation can be described by Wien's displacement law, which states that the wavelength for maximum emission of energy ($\lambda_{\max}$) times the temperature of the emitting body (T) equals $2.9 \times 10^6 \text{ nm}^\circ\text{K}$ (p. 174). Since the temperature of the surroundings is generally near 300°K , $\lambda_{\max}$ for radiation from them is close to $(2.9 \times 10^6 \text{ nm}^\circ\text{K})/(300^\circ\text{K})$, or $10,000 \text{ nm}$, which is $10 \mu\text{m}$. Thus, the emission of "thermal" radiation from the surroundings occurs predominantly at wavelengths far into the infrared.

Most of the thermal radiation from the sky comes from H_2O , CO_2 , and other molecules in the atmosphere which emit considerable radiation from 5 to $8 \mu\text{m}$ and above $13 \mu\text{m}$. Moreover, the concentration of these gases varies, so that the effective temperature of the sky (T^{sky}), as judged from its radiation, also varies (to be considered shortly). For instance, clouds contain much water in the form of vapor, droplets, and/or crystals, and this leads to a substantial emission of infrared radiation, so that T^{sky} can be as high as 280°K on a cloudy day or night. On the other hand, a dry cloudless dust-free atmosphere might have a T^{sky} as low as 200°K (see Munn or Robinson). Since effective sky temperatures are below 300°K , thermal radiation from the sky generally occurs at wavelengths shifted even further into the IR than those of the surroundings, i.e., the soil, rocks, other leaves, and plants.

We will now consider the amount of thermal radiation absorbed by a leaf. We will suppose that the infrared radiation from the surroundings, acting like a planar source at an effective temperature of T^{sur} , is incident on the lower surface of the leaf, and the upper surface of the leaf is exposed to the sky, which acts like a planar source with an effective temperature of T^{sky} (Fig. 7.6). In Chapter 6 we introduced the Stefan-Boltzmann law, which indicates that the amount of radiation emitted by a body depends markedly on its temperature (Eq. 6.17, maximum energy radiated per unit area $= \sigma T^4$). The Stefan-Boltzmann law predicts the maximum rate of energy radiation, i.e., as emitted by a perfect radiator, the so-called black-body (pp. 173, 295). Here we will use "effective temperature" in the sense that the actual energy radiated per unit area equals $\sigma(T_{\text{effective}})^4$. T^{sky} , for instance, is not the temperature we would measure at some particular location in the sky; however, $\sigma(T^{\text{sky}})^4$ does indicate the actual amount of radiant energy from the sky. Employing the Stefan-Boltzmann law, with effective temperatures to give the radiation emitted by the surroundings and

the sky, the IR absorbed by a leaf is

$$\begin{aligned} &\text{infrared radiation absorbed} \\ &\text{per unit leaf area} = a_{\text{IR}}\sigma[(T^{\text{sur}})^4 + (T^{\text{sky}})^4] \end{aligned} \quad (7.25)$$

where the absorptivity a_{IR} is the fraction of the energy of the incident infrared radiation absorbed by the leaf.

Emitted infrared radiation. Thermal radiation is also *emitted* by a leaf. Such radiation occurs at wavelengths far into the IR, since leaf temperatures, like those of its surroundings, are near 300°K. This is illustrated in Figure 7.7, where the emission of radiant energy from a leaf at 25° is plotted both in terms of wavelength and wave number.* The wave-number scale makes it easier to illustrate in the same figure the spectral distribution of radiation from both sun and leaf. The λ_{max} for sunlight is in the visible region; for the leaf it is in the infrared near 10 μm . Figure 7.7 also indicates that nearly all of the thermal radiation emitted by a leaf has wave numbers less than 5000 cm^{-1} , which corresponds to IR wavelengths greater than 2 μm .

We will express the IR emitted by a leaf at a temperature T^{leaf} using the Stefan-Boltzmann law (Eq. 6.17), which describes the maximum rate of radiation. For the general emission case we can incorporate a coefficient of radiation. For the general emission case we can incorporate a coefficient known as the emissivity, e , which takes on its maximum value of unity for a perfect, or blackbody radiator.† Since infrared radiation is emitted by both sides of a leaf (cf. Fig. 7.6), the factor 2 is necessary to describe its energy loss by thermal radiation:

$$\text{infrared radiation emitted per unit leaf area} = 2e_{\text{IR}}\sigma(T^{\text{leaf}})^4 \quad (7.26)$$

Like the other flux relations in this chapter, Equation 7.26 is expressed on the basis of unit area for one side of a leaf.

Values for a , a_{IR} , and e_{IR} . The parameters a , a_{IR} , and e_{IR} obviously help determine the energy balance of a leaf. We will first consider how the absorptivity of a leaf depends on wavelength and then discuss its emissivity for infrared radiation.

* Wave number, which we introduced in Problems 4.2 and 4.6, is the frequency of the radiation divided by the velocity of light, i.e., ν/c , which is $1/\lambda_{\text{vacuum}}$ by Equation 4.2.

† The actual energy radiated/unit area equals $e\sigma(T_{\text{actual}})^4$, which is the same as $\sigma(T_{\text{effective}})^4$. We will use emissivities and actual temperatures to describe the energy radiated by leaves; effective temperatures are usually employed for thermal radiation from the surroundings and the sky, since their temperatures are difficult to measure or are actually hypothetical.

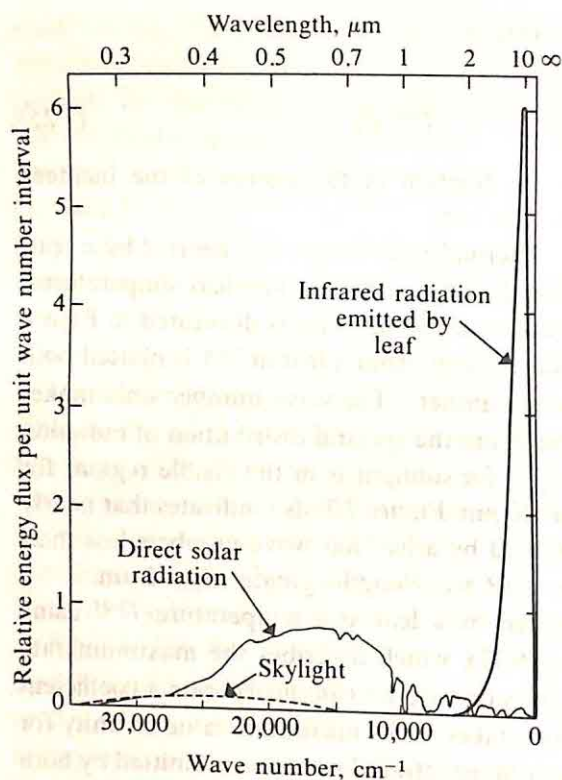


Figure 7.7

Wave number and wavelength distributions for direct solar radiation, skylight, and the radiation emitted by a leaf at 25° . The areas under the curves indicate the total energy radiated, e.g., S_{direct} here is $1.2 \text{ cal/cm}^2\text{-min}$, and $2e_{\text{IR}}\sigma(T^{\text{leaf}})^4$ is $1.3 \text{ cal/cm}^2\text{-min}$.

Figure 7.8 indicates that a_{λ} , the value of the absorptivity at a particular wavelength, varies considerably with the spectral region. For example, a_{λ} averages about 0.8 in the visible (0.4 to $0.7 \mu\text{m}$). Such relatively high fractional absorption by a leaf is due mainly to the photosynthetic pigments that we have already discussed. Figure 7.8 indicates that a_{λ} is rather small from just above $0.7 \mu\text{m}$ to nearly $1.2 \mu\text{m}$. This is quite important for minimizing the energy input into a leaf, since much global radiation occurs in this interval of the infrared. The fraction of radiation absorbed becomes essentially unity for infrared radiation beyond $2 \mu\text{m}$. This does not lead to excessive heating of the leaves from absorption of global radiation, since very little radiant emission from the sun occurs beyond $2 \mu\text{m}$ (cf. Fig. 7.7).

In our equations we have used two absorptivity coefficients, a (in Eq. 7.24) and a_{IR} (in Eq. 7.25). In contrast to a_λ , these coefficients represent absorptivities for a particular wavelength range. For example, a refers to the fraction of the incident solar energy absorbed (the wavelength distributions for direct sunlight and skylight are presented in Fig. 7.7). For most leaves, a is between 0.5 and 0.7, with 0.6 being a typical value. Figure 7.8 shows that nearly all of the IR radiation beyond $2\text{ }\mu\text{m}$ is absorbed by a leaf. In fact, a_{IR} for leaves is usually from 0.95 to 0.97, and we will use a value of 0.96 for purposes of calculation (see Gates 1965 and Gates 1970).

Next, let us compare a_{IR} with e_{IR} . Since emission of radiation is simply the reverse of its absorption, we should not be surprised that the same sort of considerations which apply to absorption of electromagnetic radiation (see Ch. 4) also apply to its emission. A good absorber of radiation is also a good emitter. In more precise language, the absorptivity a_λ equals the emissivity e_λ when they refer to the same wavelength—for a blackbody, $a_\lambda = e_\lambda = 1.00$ at all wavelengths (see Kreith). Since radiation from the

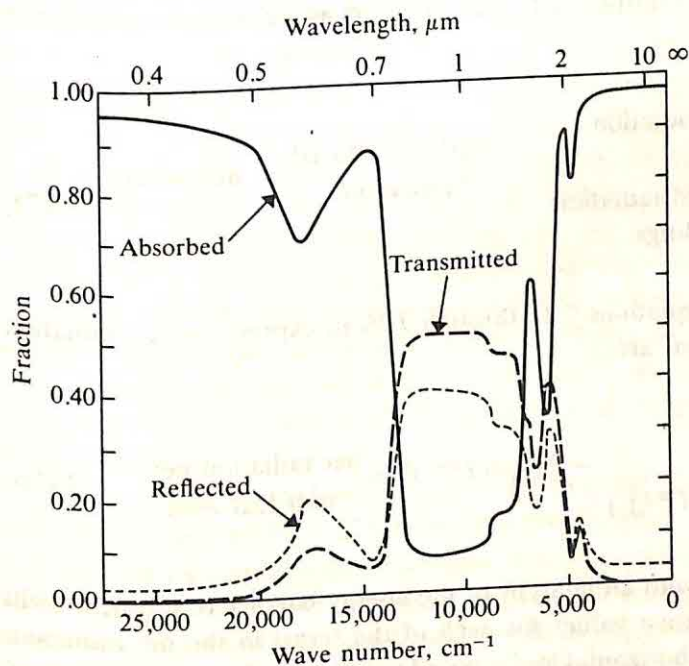


Figure 7.8

Representative values for the fractions of radiation absorbed, transmitted, and reflected by a leaf, as a function of wave number and wavelength. The sum $a_\lambda + r_\lambda + t_\lambda$ is unity. (See Gates 1965, Gates 1970, and Woolley.)

surroundings and the sky occurs at essentially the same region of the infrared as that emitted by a leaf, e_{IR} is about the same as a_{IR} , e.g., 0.96.

We will conclude with two comments. First, the amount of radiation emitted by a blackbody per unit wavelength interval is proportional to $\lambda^{-4}/(e^{hc/\lambda kT} - 1)$, as predicted by Planck's radiation formula (p. 173). When we multiply such maximum radiation by e_{λ} at each of the wavelengths involved, we obtain the actual spectral distribution of the emitted thermal radiation. Second, the absorptivity a_{λ} is related to the absorption coefficient ϵ_{λ} introduced in Chapter 4. Equation 4.15 indicates that $\log(I_o/I_f)$ equals $\epsilon_{\lambda}bc$, where b is the optical path length, c is the concentration of the absorbing species, I_o is the incident intensity, and I_f is the intensity of the emergent beam when only absorption takes place (i.e., in the absence of reflection and scattering). The fraction of radiation absorbed at a particular wavelength, $(I_o - I_f)/I_o$, is the absorptivity a_{λ} . Thus, a_{λ} equals $1 - I_f/I_o$, which is $1 - 10^{-\epsilon_{\lambda}bc}$ by Equation 4.15.

Net radiation. We have now considered each of the terms that involve radiation in the energy balance of a leaf (Eq. 7.23). It is often convenient to single out these quantities and to refer to them as the *net radiation* balance for the leaf:

$$\begin{array}{rcl} \text{absorbed solar radiation} & & \\ + & - & \text{emitted infrared} \\ \text{absorbed infrared radiation} & \text{radiation} & = \text{net radiation} \\ \text{from surroundings} & & \end{array} \quad (7.27a)$$

We can also use Equations 7.24 through 7.26 to express the net radiation balance per unit leaf area:

$$\begin{array}{rcl} a(1 + r)S & & \\ + & - & 2e_{\text{IR}}\sigma(T^{\text{leaf}})^4 = \text{net radiation per} \\ a_{\text{IR}}\sigma[(T^{\text{sur}})^4 + (T^{\text{sky}})^4] & & \text{unit leaf area} \end{array} \quad (7.27b)$$

Before continuing with an analysis of the energy balance of a leaf, we will examine representative values for each of the terms in the net radiation.

Let us consider a horizontal leaf exposed to full sunlight at sea level where the global radiation (S) is $1.20 \text{ cal/cm}^2\text{-min}$. We will assume that the absorptivity of the leaf for global radiation (a) equals 0.60, and that the reflectivity (r) is 0.20. By Equation 7.24, the direct plus the reflected sunlight, cloudlight, and skylight absorbed by the leaf is $(0.60)(1.00 + 0.20)(1.20 \text{ cal/}$

$\text{cm}^2\text{-min}$), or $0.86 \text{ cal/cm}^2\text{-min}$. To calculate the infrared radiation absorbed by the leaf, we will let a_{IR} be 0.96, the temperature of the surroundings be 20° , and the sky temperature be -30° . Using Equation 7.25, with a Stefan-Boltzmann constant of $8.13 \times 10^{-11} \text{ cal cm}^{-2} \text{ min}^{-1} \text{ K}^{-4}$ (App. II), the absorbed IR per unit area of the leaf is $(0.96)(8.13 \times 10^{-11} \text{ cal cm}^{-2} \text{ min}^{-1} \text{ K}^{-4}) [(293^\circ\text{K})^4 + (243^\circ\text{K})^4]$, or $0.84 \text{ cal/cm}^2\text{-min}$. Hence, the total energy load on the leaf by radiation is $(0.86) + (0.84)$, or $1.70 \text{ cal/cm}^2\text{-min}$.

In the present case the rate of energy input per unit leaf area ($1.70 \text{ cal/cm}^2\text{-min}$) is nearly the size of the solar constant ($2.00 \text{ cal/cm}^2\text{-min}$). The total energy load on the leaf is about equally contributed here by the various forms of radiation from the sun ($0.86 \text{ cal/cm}^2\text{-min}$) and IR radiation from the surroundings plus the sky ($0.84 \text{ cal/cm}^2\text{-min}$). Since the sky generally has a much lower effective temperature for radiation than the surroundings, the upper surface of an exposed leaf usually receives less IR than the lower one: $a_{\text{IR}}\sigma(T^{\text{sky}})^4$ here is $(0.96)(8.13 \times 10^{-11})(243)^4$, or $0.26 \text{ cal/cm}^2\text{-min}$, while the IR absorbed by the lower surface of the leaf, $a_{\text{IR}}\sigma(T^{\text{sur}})^4$, is $0.58 \text{ cal/cm}^2\text{-min}$. The total radiation load on an exposed leaf generally does not change by more than $0.4 \text{ cal/cm}^2\text{-min}$ however much the leaf angle varies, since the IR, the scattered, and the reflected radiation received by a leaf come from all angles. However, leaf angle can have important implications for the adaptation of certain plants to their environment.

To estimate the infrared radiation emitted by a leaf, we will let e_{IR} be 0.96 and the leaf temperature be 25° . By Equation 7.26, IR emitted $= 2e_{\text{IR}}\sigma(T^{\text{leaf}})^4$, we calculate that the energy loss by thermal radiation is $(2)(0.96)(8.13 \times 10^{-11} \text{ cal cm}^{-2} \text{ min}^{-1} \text{ K}^{-4})(298^\circ\text{K})^4$, or $1.24 \text{ cal/cm}^2\text{-min}$. In this case, the IR emitted by both sides of the exposed leaf is about 50% greater than the leaf's absorption of either infrared ($0.84 \text{ cal/cm}^2\text{-min}$) or solar radiation ($0.86 \text{ cal/cm}^2\text{-min}$). (Leaves shaded by other leaves are not fully exposed to T^{sky} , and for them IR emitted can be approximately equal to IR absorbed.)

The net radiation balance for our exposed leaf is $(1.70) - (1.24)$, or $0.46 \text{ cal/cm}^2\text{-min}$ (cf. Eq. 7.27). As we will discuss below, such excess energy is dissipated by conduction, convection, and the evaporation of water accompanying transpiration. However, most of the energy input into a leaf is actually balanced by the emission of infrared radiation. The IR emitted in the present case, for example, amounts to $(1.24/1.70)(100)$, or 73% of the energy input from all sources of incident radiation.

Examples for radiation terms. The radiation terms in the previous section are summarized in Table 7.3, top line. Since many conditions affect these terms, let us now consider some other examples. S at noon on a cloudless

Table 7.3

Representative values for the various terms involved in the net radiation balance of an exposed leaf. Equation 7.27 is used, assuming that a is 0.60 (except where indicated), r is 0.20, and that both a_{IR} and e_{IR} are 0.96. (See text for interpretations.)

Condition	Global radiation, S (cal/cm ² -min)	Absorbed solar radiation, $a(1 + r)S$ (cal/cm ² -min)	Temperature of surroundings	Sky temperature	Absorbed infrared, $a_{\text{IR}}\sigma[(T_{\text{sur}})^4 + (T_{\text{sky}})^4]$ (cal/cm ² -min)	Leaf temperature	Emitted infrared, $2 e_{\text{IR}}\sigma(T_{\text{leaf}})^4$ (cal/cm ² -min)	Net radiation (cal/cm ² -min)
Sea level on cloudless day	1.20	0.86	20°	-30°	0.84	25°	1.24	0.46
2000 m on cloudless day	1.50	1.08	36°	-35°	0.96	44°	1.58	0.46
Silvery leaf ($a = 0.50$) at 2000 m on cloudless day	1.50	0.90	36°	-35°	0.96	35°	1.40	0.46
Sea level on cloudy night	0.00	0.00	1°	1°	0.88	1°	0.88	0.00
Sea level on cloudless night	0.00	0.00	1°	-30°	0.71	-13°	0.71	0.00
	0.00	0.00	1°	-30°	0.71	-1°	0.86	-0.15

day at an elevation of 2000 m might be $1.50 \text{ cal/cm}^2\text{-min}$. Because of the higher incident global radiation at 2000 m, the leaf will absorb $0.22 \text{ cal/cm}^2\text{-min}$ more direct, scattered, and reflected solar radiation than our example at sea level. The effective temperature of the sky is generally slightly lower at the higher elevation (-35° vs. -30° in Table 7.3). We will consider a high desert where the surroundings are considerably warmer than for our example at sea level (e.g., 36° vs. 20°). There is, then, $0.12 \text{ cal/cm}^2\text{-min}$ more IR absorbed by the leaf at 2000 m. The total energy input is $2.04 \text{ cal/cm}^2\text{-min}$, which is $0.34 \text{ cal/cm}^2\text{-min}$ greater than at sea level (cf. Table 7.3). For the net radiation to be the same in the two cases ($0.46 \text{ cal/cm}^2\text{-min}$), the leaf at the higher altitude must emit $0.34 \text{ cal/cm}^2\text{-min}$ more thermal radiation than the leaf at sea level. As Table 7.3 indicates, this could be accomplished if T^{leaf} for the leaf at 2000 m were 19° higher than the one at sea level.

Certain plants have silvery or shiny leaves, which increases the amount of solar radiation that is reflected. The fraction of S reflected by the leaf may increase from typical values of 0.1–0.2 (cf. Fig. 7.8) to 0.3 for silvery leaves, with an accompanying reduction in the absorptivity from 0.6 to 0.5 (see Gates 1970). This reduction in absorptivity can have a marked influence on T^{leaf} . Other conditions remaining the same, a reduction of the absorptivity a by only 0.1 can cause the leaf temperature to go from 44° to 35° (Table 7.3). This can be an advantage to a desert plant exposed to high incident radiation, since the rate of photosynthesis is generally much higher at a leaf temperature of 35° than at 44° .

Let us next consider the effect of clouds on leaf temperature at night. On a clear night at sea level the effective sky temperature might be -30° , while for a heavy cloud cover it could be 1° because of the IR emitted by the clouds (Table 7.3). If the temperature of the surroundings were 1° in both cases, the infrared absorbed by the leaf, $a_{\text{IR}}\sigma[(T^{\text{sur}})^4 + (T^{\text{sky}})^4]$, would be $0.17 \text{ cal/cm}^2\text{-min}$ lower on the clear night because of less IR from the cloudless sky (Table 7.3). For there to be no net gain or loss of energy by a leaf from all forms of radiation, i.e., $a_{\text{IR}}\sigma[(T^{\text{sur}})^4 + (T^{\text{sky}})^4] = 2e_{\text{IR}}\sigma(T^{\text{leaf}})^4$, the leaf temperature would have to be 1° on the cloudy night and -13° on the clear one (Table 7.3). Thus, on the clear night with surroundings at 1° , T^{leaf} would be considerably below freezing, and heat would be conducted from the surroundings to the leaf, raising its temperature to perhaps -1° . Using this leaf temperature, we can calculate that there would be a net energy loss by radiation of $0.15 \text{ cal/cm}^2\text{-min}$ on the clear night (see Table 7.3). A plant on a cloudless night may therefore freeze even though the temperatures of both air and surroundings are above 0° , since

the excess of IR emitted over that absorbed can lower the leaf temperature below the freezing point. Such freezing of leaves on clear nights is a severe problem for certain agricultural crops (see Geiger and Schwintzer).

Heat conduction and convection. Heat is conducted from one body to a cooler one in contact with it by molecular and/or electronic collisions. We will assume here that the leaf and its petiole have the same temperature, and that in general a temperature difference exists across the unstirred air layer adjacent to the leaf surface. Heat can be *conducted* across this air layer by the random thermal collisions of the gas molecules. Heat convection, on the other hand, involves turbulent movement of a fluid, brought about for example by differences in pressure. There are two types of convection: *free* and *forced*. Free (natural) convection occurs when the heat transferred from a leaf causes the air outside the unstirred layer to expand and thus to decrease in density; this more buoyant warmer air then moves upward and thereby moves heat away from the leaf. Wind can also remove the heated air outside the unstirred layer, an instance of forced convection. As wind speed increases, more and more of the heat is dissipated by forced convection. However, even at a wind speed of only 10 cm/sec, which is essentially the lowest occurring naturally (p. 306), forced convection dominates free convection as a means of heat loss from most leaves. We will therefore assume that heat is *conducted* across the unstirred layer adjacent to the leaf and then removed by forced *convection* in the surrounding turbulent air.

For the one-dimensional case, heat flow by conduction equals $-K \partial T / \partial x$, where K is known as the thermal conductivity coefficient and $\partial T / \partial x$ is the temperature gradient. Since heat can be conducted across the unstirred layers on either side of a leaf, we need to incorporate the factor 2 to describe the total heat flux by conduction per unit area of one side of the leaf. (For convenience, we will assume that the two unstirred air layers are of equal thickness, δ^a .) The amount of heat conducted across the unstirred air layers and convected away from a leaf per unit time and area therefore is

$$\begin{aligned} J_H^C &= -2K^{\text{air}} \frac{\partial T}{\partial x} \\ &\cong 2K^{\text{air}} \frac{(T^{\text{leaf}} - T^{\text{ta}})}{\delta^a} \end{aligned} \quad (7.28)$$

where J_H^C is the rate of heat conduction per unit area (e.g., cal/cm²-min), K^{air} is the thermal conductivity coefficient of air, T^{leaf} is the leaf temperature, and T^{ta} is the temperature of the turbulent air outside an unstirred layer of thickness δ^a . Since heat is conducted from the solid surface of the leaf across the adjacent unstirred air, it does not matter whether the stomates

are opened or closed. In other words, we have a planar source at a specific temperature (T^{leaf}) from which heat is conducted across the unstirred air layer to the outside turbulent air (at T^{ta}). The heat flow in Equation 7.28 is considered positive when it goes from the leaf to the surrounding air. Of course, heat is conducted into the leaf when T^{leaf} is less than T^{ta} .

Values of the thermal conductivity coefficient of dry air at various temperatures are given in $(\text{cal}/\text{cm}^2\text{-min})/(\text{°}/\text{cm})$ in Appendix II. Since the conduction of heat in a gas phase is based on the random thermal motion of the molecules, the composition of air can influence the value of K^{air} . Our main concern here is with the effect of water vapor content on K^{air} , an influence which is relatively small at the usual temperatures encountered by leaves. For instance, K^{air} at 25° is only 1% less for 100% relative humidity than it is for dry air. Air can hold considerably more water vapor at 50° , where K^{air} is 4% lower for water-saturated air than that of 0% relative humidity. Use of the values of K^{air} in Appendix II is generally adequate for most calculations of heat conduction into or out of leaves.

Examples of heat conduction. Let us now calculate the heat conduction expected from a leaf with an unstirred air layer 1.3 mm thick on each side and a temperature of 25° when the surrounding air is at 21° . K^{air} is $3.4 \times 10^{-3} (\text{cal}/\text{cm}^2\text{-min})/(\text{°}/\text{cm})$ for air from 21° to 25° (App. II). Using Equation 7.28, we calculate that J_H^{c} is $(2)[3.4 \times 10^{-3} (\text{cal}/\text{cm}^2\text{-min})/(\text{°}/\text{cm})](25^{\circ} - 21^{\circ})/(0.13 \text{ cm})$, or $0.21 \text{ cal}/\text{cm}^2\text{-min}$. A leaf at 25° at sea level on a sunny day could have a net radiation balance of $0.46 \text{ cal}/\text{cm}^2\text{-min}$ (cf. Table 7.3, top line). In the present case just under half ($0.21 \text{ cal}/\text{cm}^2\text{-min}$) of this energy input from radiation is dissipated by conduction of heat across the unstirred air layer on each side of the leaf, followed by forced convection in the surrounding turbulent air.

A leaf with a thick unstirred layer can have a temperature quite different from that of the air, since air is a relatively poor conductor of heat. Specifically, K^{air} is relatively low compared with the thermal conductivity coefficients for liquids and most solids. A large leaf in a low wind might have an unstirred layer 0.40 cm thick (see Table 7.1 or Eq. 7.3 for wind velocities and leaf sizes implied by this). If J_H^{c} and K^{air} are the same as in the previous paragraph, where the difference between leaf and turbulent air temperatures is 4° when the unstirred layer is 0.13 cm thick, then $T^{\text{leaf}} - T^{\text{ta}}$ would be $(0.40 \text{ cm}/0.13 \text{ cm})(4^{\circ})$, or 12° for a δ^{a} of 0.40 cm. Thus, the combination of large leaves and low wind velocities favors a large drop in temperature across the unstirred air layers. On the other hand, a small leaf in a moderate wind could have a δ^{a} of 0.024 cm. For the same J_H^{c} and K^{air} as above, $T^{\text{leaf}} - T^{\text{ta}}$ would be $(0.024 \text{ cm}/0.13 \text{ cm})(4^{\circ})$, or only 1° for this thin unstirred layer. Hence, small leaves tend to have temperatures quite close to

that of the air, especially at moderate to high wind velocities. This close coupling between leaf and air temperatures for small leaves can keep the leaf temperature low enough for optimal photosynthesis (often 30° to 35°) in hot sunny climates. Also, the lower the leaf temperature, the lower is the concentration of water vapor in the pores of the cell walls of mesophyll cells, and consequently less water would then tend to be lost in transpiration, an important consideration in arid and semi-arid regions.

We can apply the same heat conduction analysis to the flat pads on prickly-pear cactus, although the pads are modified stems, not leaves. Let us consider a pad 25 cm across, exposed to the sun on a cloudless day in the high desert (e.g., 2000 m). The radiation terms for this case are presented in Table 7.3, line 2. The "leaf" temperature is 44° , and the net radiation represents a heat load of $0.46 \text{ cal/cm}^2\text{-min}$. Cacti are CAM plants (p. 342), so the stomates tend to be *closed* during the day. Therefore, transpiration does not lead to much cooling of the pads. Thus, essentially all of the net radiation on a cactus pad during the daytime is balanced by heat conduction across the unstirred air layers to the surrounding turbulent air. (Here we are neglecting possible heat conduction down the stem.) For a typical ambient wind velocity of 100 cm/sec, Table 7.1 indicates that the unstirred layer is 0.20 cm thick when $l_{(\text{cm})}^{\text{leaf}}$ is 25 cm. We can estimate the temperature of the turbulent air surrounding this pad by Equation 7.28, $J_H^C \cong 2K^{\text{air}}(T^{\text{leaf}} - T^{\text{ta}})/\delta^a$, where K^{air} is $3.5 \times 10^{-3} \text{ (cal/cm}^2\text{-min)/}(\circ/\text{cm})$ for air temperatures near 40° (App. II). In particular, T^{ta} equals $T^{\text{leaf}} - J_H^C \delta^a / (2K^{\text{air}})$, or $44^\circ - (0.46 \text{ cal/cm}^2\text{-min})(0.20 \text{ cm}) / \{(2)[3.5 \times 10^{-3} \text{ (cal/cm}^2\text{-min)/}(\circ/\text{cm})]\}$, which is 31° . In this case, where the net radiation ($0.46 \text{ cal/cm}^2\text{-min}$) is balanced by heat conduction across the unstirred layer, the cactus pad is 13° warmer than the surrounding turbulent air, a condition which can in fact occur on a sunny day in the high desert.

Heat flux accompanying transpiration. Evaporation of water is of course a cooling process. Since water evaporates at the air-liquid interfaces along the pores in the cell walls of mesophyll cells, transpiration leads to a heat loss by a leaf (cf. Eq. 7.23).

The flux of water vapor out of a leaf during transpiration is J_{wv} . If we multiply the amount of water leaving per unit time and leaf area (J_{wv}) by the energy necessary to evaporate a unit amount of water at the temperature of the leaf (H_{vap}), we obtain the heat flux accompanying transpiration, J_H^T :

$$\begin{aligned} J_H^T &= J_{wv} H_{\text{vap}} \\ &= \frac{H_{\text{vap}} \Delta c_{wv}^{\text{total}}}{R_{wv}^{\text{total}}} = \frac{H_{\text{vap}} (c_{wv}^{\text{m}} - c_{wv}^{\text{ta}})}{R_{wv}^{\text{total}}} \end{aligned} \quad (7.29)$$

where Equation 7.12 ($J_{wv} = \Delta c_{wv}^{\text{total}} / R_{wv}^{\text{total}}$) is used to express J_{wv} in terms

of a concentration drop and a resistance. By Equation 7.11, $\Delta c_{wv}^{\text{total}}$ equals c_{wv}^m , the concentration of water vapor in the cell wall pores of mesophyll cells at the temperature of the leaf, minus c_{wv}^{ta} , the water vapor concentration in the turbulent air surrounding the leaf.

How much of the heat load on a leaf is dissipated by the evaporation of water during transpiration? On p. 323 we indicated that J_{wv} could be $0.41 \mu\text{mole}/\text{cm}^2\text{-sec}$ for the leaf of a typical mesophyte. In Chapter 2 we noted that water has a high heat of vaporization, e.g., 10.5 kcal/mole at 25° (values at other temperatures are given in App. II). By Equation 7.29, J_H^T would then be $(0.41 \times 10^{-6} \text{ mole}/\text{cm}^2\text{-sec})(60 \text{ sec/min})(10.5 \times 10^3 \text{ cal/mole})$, or $0.26 \text{ cal}/\text{cm}^2\text{-min}$. For the leaf described in the top line of Table 7.3, a heat loss of $0.26 \text{ cal}/\text{cm}^2\text{-min}$ by evaporation would dissipate slightly over half of the net radiation ($0.46 \text{ cal}/\text{cm}^2\text{-min}$), the rest of the energy input being removed by heat conduction followed by forced convection. The heat loss accompanying transpiration can help prevent excessive leaf temperatures during the daytime.

Heat flux for dew or frost formation. So far, we have regarded c_{wv}^m as greater than c_{wv}^{ta} , in which case there is a net loss of water from the leaf and a consequent heat dissipation. However, when the turbulent air is warmer than the leaf, the water vapor concentration in the turbulent air may be greater than that in the leaf, leading to a net diffusion of water vapor toward the leaf. This can cause c_{wv} at the leaf surface to increase and it may reach c_{wv}^* , the saturation value, especially if the stomates are closed (which would limit the diffusion of water vapor into the leaf). If c_{wv}^{ta} is greater than this c_{wv}^* , dew—or frost, if the leaf temperature is below freezing—can form as water vapor diffuses toward the leaf and then condenses onto its surface, which is cooler than the turbulent air.

Condensation of water vapor leads to a heat *gain* by a leaf. Since water condensation is the reverse of its evaporation, the heat gain per unit amount of water condensed is the heat of vaporization of water at the temperature of the leaf, H_{vap} . If the condensation is on the leaf surface, the diffusion step involves only the unstirred air layers, each of which has a resistance R_{wv}^a . We should then modify Equation 7.29 to the following form to describe the heat flux accompanying such water vapor condensation:

heat gain by dew formation per unit leaf area

$$= \frac{2H_{\text{vap}}(c_{wv}^{\text{ta}} - c_{wv}^{\text{leaf}*})}{R_{wv}^a} \quad (7.30)$$

where $c_{wv}^{\text{leaf}*}$ is the saturation concentration of water vapor at the temperature of the leaf. The factor 2 is necessary because water vapor diffuses toward

the leaf across the unstirred air layer on each side. (As usual, we will assume that these layers are of equal average thickness and hence equal resistance.)

The temperature to which the turbulent air must be reduced at constant pressure in order for it to become saturated with water vapor is known as the *dew point*, or dew temperature, $T^{\text{ta}}_{\text{dew point}}$. When T^{leaf} is below $T^{\text{ta}}_{\text{dew point}}$, the turbulent air contains more water vapor ($c_{\text{wv}}^{\text{ta}}$) than the air at the leaf surface can hold ($c_{\text{wv}}^{\text{leaf*}}$). Water vapor then diffuses toward the leaf, and this can lead to dew formation. If T^{leaf} is below 0° and less than $T^{\text{ta}}_{\text{dew point}}$, the water which condenses onto the leaf surface will freeze. Under such conditions we should replace H_{vap} in Equation 7.30 by the heat of sublimation (H_{sub}) at that particular leaf temperature in order to describe the heat gain by frost formation.

Examples of frost and dew formation. As an example of nighttime frost formation, we will consider a leaf with unstirred layers 1.3 mm thick and a temperature of -1° when the surrounding air is at 1° and 93% relative humidity. The resistance of an unstirred layer to the diffusion of water vapor (R_{wv}^{a}) equals $\delta^{\text{a}}/D_{\text{wv}}$ by Equation 7.2. Since D_{wv} is about $0.23 \text{ cm}^2/\text{sec}$ at 0° and δ^{a} is 0.13 cm here, R_{wv}^{a} is $(0.13 \text{ cm})/(0.23 \text{ cm}^2/\text{sec})$, or 0.57 sec/cm . H_{sub} is 12.2 kcal/mole at -1° (App. II), $c_{\text{wv}}^{\text{ta}}$ is $(0.93)(0.29 \text{ } \mu\text{mole}/\text{cm}^3)$, or $0.27 \text{ } \mu\text{mole}/\text{cm}^3$ at 1° , while c_{wv}^* is $0.25 \text{ } \mu\text{mole}/\text{cm}^3$ at -1° (App. II). By Equation 3.30, the heat gain by frost formation is then $(2)(12.2 \times 10^3 \text{ cal/mole})[(0.27 \times 10^{-6} \text{ mole}/\text{cm}^3) - (0.25 \times 10^{-6} \text{ mole}/\text{cm}^3)]/[(0.57 \text{ sec/cm})(1 \text{ min}/60 \text{ sec})]$, or $0.05 \text{ cal}/\text{cm}^2\text{-min}$. Since T^{leaf} is less than T^{ta} here, heat is conducted *into* the leaf from the air. By Equation 7.28, $J_H^{\text{c}} \cong 2K^{\text{air}}(T^{\text{leaf}} - T^{\text{ta}})/\delta^{\text{a}}$, where K^{air} is $3.25 \times 10^{-3} \text{ (cal}/\text{cm}^2\text{-min})/(\text{ }^\circ/\text{cm})$ at 0° (App. II), the heat conduction is $(2)[3.25 \times 10^{-3} \text{ (cal}/\text{cm}^2\text{-min})/(\text{ }^\circ/\text{cm})](-1^\circ - 1^\circ)/(0.13 \text{ cm})$, or $-0.10 \text{ cal}/\text{cm}^2\text{-min}$, where the minus sign indicates that heat is conducted into the leaf. The bottom line of Table 7.3 indicates that this leaf can lose $0.15 \text{ cal}/\text{cm}^2\text{-min}$ by net radiation. Thus, the heat inputs from frost formation ($0.05 \text{ cal}/\text{cm}^2\text{-min}$) and heat conduction ($0.10 \text{ cal}/\text{cm}^2\text{-min}$) just balance the energy loss by net radiation.

How long would it take to form a layer of frost 0.1 mm thick on each side of a leaf? The heat flux for frost formation equals $J_{\text{wv}}H_{\text{sub}}$. Using 680 cal/g for H_{sub} at -1° (App. II), J_{wv} in the previous example is $(0.05 \text{ cal}/\text{cm}^2\text{-min})/(680 \text{ cal/g})$, or $7 \times 10^{-5} \text{ g}/\text{cm}^2\text{-min}$. Since ice has a density of $0.917 \text{ g}/\text{cm}^3$, frost would be building up at a rate of $(7 \times 10^{-5} \text{ g}/\text{cm}^2\text{-min})/(0.917 \text{ g}/\text{cm}^3)$, or $8 \times 10^{-5} \text{ cm}/\text{min}$. A layer 0.02 cm thick (total for both sides of the leaf) would thus form in $(0.02 \text{ cm})/(8 \times 10^{-5} \text{ cm}/\text{min})$, or 250 min, about 4 hr. Dew formation can also be as high as a few tenths of a millimeter per night, an important source of water for leaves in certain regions for much of the year (see Levitt).

Dew or frost formation is favored on cloudless nights and when the relative humidity of the surrounding turbulent air is close to 100%. As we mentioned above, T^{leaf} tends to be further from T^{ta} for large leaves than for small ones. On a cloudless night when T^{leaf} is less than T^{sur} and T^{ta} (cf. Table 7.3), the larger exposed leaves will generally dip below $T^{\text{ta}}_{\text{dew point}}$ sooner than the small ones, hence dew or frost tends to form on the large leaves first. For convenience, we have assumed that the leaf has a uniform temperature. However, the unstirred layer tends to be thinner at the edges of a leaf, so that T^{leaf} is somewhat closer to T^{ta} at the edges than it is at the center. Thus, dew or frost generally forms first at the center of the leaf, where δ^a is larger and the temperature slightly lower than at the leaf edges. Also, there can be a small temperature gradient across the thickness of a leaf. Since only the upper surfaces of the uppermost leaves are fully exposed to the low T^{sky} , the upper surfaces of such leaves actually have the lowest temperature, and dew or frost tends to form there first.

Shaded leaves within plant communities. So far in this chapter we have been primarily concerned with the energy and gaseous fluxes of exposed leaves. We can easily extend our analysis to include leaves shaded by overlying ones.

As a convenient example, let us consider a shaded leaf at the same temperature as its surroundings. As we indicated on p. 353, the infrared radiation absorbed by such a leaf would be the same as the IR emitted by it (when $a_{\text{IR}} = e_{\text{IR}}$ and $T^{\text{leaf}} = T^{\text{sur}}$). The net radiation would then be due solely to the various forms of solar radiation that reach the leaf. Since the transmission by leaves is fairly high from 0.7 to $2 \mu\text{m}$ (Fig. 7.8), much of the solar radiation reaching a shaded leaf would be in a region not useful for photosynthesis. The leaf absorptivity in this range is lower than would be the case for the solar radiation incident on an exposed leaf (Fig. 7.8). For purposes of calculation, we will assume that S on the shaded leaf is $0.10 \text{ cal/cm}^2\text{-min}$, its absorptivity (a) is 0.30, and the reflectivity (r) of the surroundings has the rather high value of 0.4 because of considerable reflection of radiation by the other leaves within the plant community. By Equations 7.24 and 7.27, the net radiation balance is then $(0.30)(1.00 + 0.40)(0.10 \text{ cal/cm}^2\text{-min})$, or $0.04 \text{ cal/cm}^2\text{-min}$.

Our shaded leaf has a rather low rate of photosynthesis because the amount of radiation in the visible region reaching it is fairly small. Moreover, the stomates generally are partially closed at the lower illumination levels, which increases the stomatal resistance and further decreases photosynthesis (cf. p. 343). Three factors tend to reduce the flux of water vapor out of a shaded leaf: the increase in stomatal resistance, which of course reduces transpiration; a lower wind velocity for a protected than an exposed leaf,

which leads to a thicker unstirred layer; and the concentration of water vapor in the turbulent air is generally higher than at the top of the plant community. Instead of a J_{wv} of $0.41 \mu\text{mole}/\text{cm}^2\text{-sec}$ for the exposed leaf of a typical mesophyte (p. 323), the flux of water vapor for the shaded leaf might be only $0.06 \mu\text{mole}/\text{cm}^2\text{-sec}$. By Equation 7.29 ($J_H^T = J_{wv} H_{\text{vap}}$), this would lead to a heat dissipation of $(0.06 \times 10^{-6} \text{ mole}/\text{cm}^2\text{-min})(60 \text{ sec}/\text{min})(10.5 \times 10^3 \text{ cal}/\text{mole})$, or $0.04 \text{ cal}/\text{cm}^2\text{-min}$. No heat is conducted across the unstirred layer on either side of a leaf at the same temperature as the surrounding air. Thus, the heat loss by water evaporation ($0.04 \text{ cal}/\text{cm}^2\text{-min}$) balances the energy gain from the net radiation absorbed by this leaf.

Of course, individual leaves vary tremendously in their physical and physiological properties, and exist in many different environments. The equations developed in this chapter can be used to describe the gaseous fluxes and energy balances of these varied situations.

Problems

7.1 A circular leaf is 0.5 mm thick and 10 cm in diameter with 7000 stomates per cm^2 . We will approximate the stomatal opening by a rectangle $6 \mu\text{m}$ by $20 \mu\text{m}$; the depth of the stomatal pore is $25 \mu\text{m}$. Assume that leaf and air temperatures are both 20° . (a) What are δ^a and R_{wv}^a if the ambient wind velocity is $2 \text{ m}/\text{sec}$? (b) What are na^{st} and the effective r^{st} ? (c) How does the magnitude of the average flux of water vapor within the stomatal pores compare with that across the unstirred air layer adjacent to the leaf? (d) What is R_{wv}^{st} ? (e) What is R_{wv}^{ias} if the effective path length in the intercellular air spaces is half of the leaf thickness? (f) Suppose that each stomate is sunken in a cylindrical cavity $50 \mu\text{m}$ across and $100 \mu\text{m}$ deep. What additional resistance to water vapor diffusion does this provide?

7.2 R_{wv}^a is $0.7 \text{ sec}/\text{cm}$, R_{wv}^{st} is $1.7 \text{ sec}/\text{cm}$, R_{wv}^c is $60 \text{ sec}/\text{cm}$, and R_{wv}^{ias} is $0.3 \text{ sec}/\text{cm}$. (a) What is R_{wv}^{total} if water vapor diffuses out only across the lower epidermis of the leaf? (b) What are the three R_{wv}^{total} 's in (a) if the cuticular pathway is ignored, if the resistance of the intercellular air spaces is ignored, and if both R_{wv}^c and R_{wv}^{ias} are ignored? (c) What is R_{wv}^{total} if the stomates in the upper epidermis are of the same size and frequency as in the lower one? (d) What is R_{wv}^{total} if 27% of J_{wv} is through the upper epidermis? (e) Suppose that the leaf temperature is 30° , the air in the cell wall pores of mesophyll cells is at 99% relative humidity, and that c_{wv}^{ta} is $15 \mu\text{g}/\text{cm}^3$. What is J_{wv} through the lower epidermis? (f) Under the conditions of (e) and ignoring cuticular transpiration, what is the drop in water vapor concentration in moles/ cm^3 along the stomatal pores?

7.3 Suppose that a shade leaf has a layer of tightly packed palisade mesophyll cells with rectangular sides which are externally $30 \mu\text{m}$ by $100 \mu\text{m}$ and with square ends $30 \mu\text{m}$ by $30 \mu\text{m}$ (the long dimension is perpendicular to the leaf surface). Suppose that there are two spherical spongy mesophyll cells ($30 \mu\text{m}$ in diameter)

under each palisade cell. We will let the cell wall thickness of mesophyll cells be $1.5 \mu\text{m}$, the mean distance from the plasmalemma to the chloroplasts be $0.1 \mu\text{m}$, and the average distance that CO_2 diffuses in the chloroplasts before reaching the photosynthetic enzymes be $0.5 \mu\text{m}$. (a) What is A^{mes}/A if essentially the entire surface area of the mesophyll cells is exposed to the intercellular air spaces? (b) Let us assume that a sun leaf on the same plant has two layers of palisade cells and half as many spongy mesophyll cells. If the dimensions of the cells are the same as for the shade leaf, what is A^{mes}/A for the sun leaf? (c) If $D_{\text{CO}_2}^{\text{cw}}$ is $5 \times 10^{-6} \text{ cm}^2/\text{sec}$, what is the maximum value for $R_{\text{CO}_2}^{\text{cw}}$ at 20° (for the shade leaf)? (d) If P_{CO_2} is $2.0 \times 10^{-2} \text{ cm/sec}$ for the plasmalemma and the chloroplast limiting membranes, what are $R_{\text{CO}_2}^{\text{pl}}$ and $R_{\text{CO}_2}^{\text{clm}}$ (shade leaf)? (e) If $D_{\text{CO}_2}^{\text{cyt}}$ and $D_{\text{CO}_2}^{\text{stroma}}$ are $1.0 \times 10^{-5} \text{ cm}^2/\text{sec}$, what are $R_{\text{CO}_2}^{\text{cyt}}$ and $R_{\text{CO}_2}^{\text{stroma}}$ (shade leaf)? Assume that the relevant partition coefficients for the various forms of CO_2 are unity. (f) What is the resistance to CO_2 diffusion from the intercellular air spaces to the photosynthetic enzymes for the sun leaf?

7.4 Suppose that $c_{\text{CO}_2}^{\text{ta}}$ is 13 nmoles/cm^3 , K_{MCO_2} is $5 \mu\text{M}$, $R_{\text{CO}_2}^{\text{ai}}$ is 0.6 sec/cm , $R_{\text{CO}_2}^{\text{leafi}}$ is 2.5 sec/cm , $R_{\text{CO}_2}^{\text{mes}}$ is 3.0 sec/cm , and $R_{\text{CO}_2}^{\text{chl}}$ is 2.5 sec/cm for a crop plant leaf which CO_2 enters only across the lower epidermis. (a) If the rate of gross photosynthesis is $1 \mu\text{mole of CO}_2 \text{ fixed/cm}^2\text{-sec}$ when $c_{\text{CO}_2}^{\text{chl}}$ is $2 \mu\text{M}$, what is V_{maxCO_2} ? (b) What is $c_{\text{CO}_2}^{\text{chl}}$ when v_{CO_2} is 90% of V_{maxCO_2} ? (c) If the rate of respiration plus photorespiration is 45% of that of gross photosynthesis, what are $R_{\text{CO}_2}^{\text{total}}$ and J_{CO_2} ? Assume that $c_{\text{CO}_2}^{\text{chl}}$ is $4 \mu\text{M}$. (d) Repeat (c) for a nonphotorespiring plant where the rate of respiration is 5% of $J_{\text{CO}_2}^{\text{ps}}$. Assume that $c_{\text{CO}_2}^{\text{chl}}$ is $2 \mu\text{M}$. (e) Let us place a small transparent bag completely around a leaf of the nonphotorespiring plant. What would $c_{\text{CO}_2}^{\text{chl}}$ be if the CO_2 concentration in the bag in the steady state were 10 ppm? Assume that all resistances and the rate of respiration are unchanged. (f) What is the concentration of carbon dioxide in the mitochondria at night for the nonphotorespiring plant? Let $R_{\text{CO}_2}^{\text{i}}$ be 8 sec/cm and assume that the rate of respiration as well as the resistances remain the same as the daytime values.

7.5 An exposed leaf at 10° has an a_{IR} and e_{IR} of 0.96, while a is 0.60 and r is 0.10. Suppose that a cloudy sky has an effective temperature for radiation of 275°K , while for a clear sky it is -40° . (a) If the absorbed IR equals the emitted IR for the leaf, what are the temperatures of the surroundings for a clear sky and for a cloudy one? (b) What are the λ_{max} 's for emission of radiant energy by the leaf and the surroundings for (a)? (c) If the global radiation is $0.98 \text{ cal/cm}^2\text{-min}$ and the temperature of the surroundings is 9° on a clear day, how much radiation is absorbed by the leaf? (d) Under the conditions of (c), what percentage of the energy input by absorbed radiation is dissipated by the emission of thermal radiation? (e) Assume that the clouds block out the sunlight and the skylight. Let S^{cloud} be $0.40 \text{ cal/cm}^2\text{-min}$ on the upper surface of the leaf, while 15% as much is reflected onto the lower surface. If the temperature of the surroundings is 9° , what is the net radiation for the leaf?

7.6 Suppose that $R_{\text{uv}}^{\text{ias}}$ is 0.2 sec/cm , $R_{\text{uv}}^{\text{sti}}$ is 2.0 sec/cm , and $R_{\text{uv}}^{\text{ai}}$ is 0.6 sec/cm

for a transpiring leaf with stomates only in the lower epidermis. (a) What is the essentially immediate effect on R_{wv}^{total} and J_{wv} of decreasing the stomatal opening fourfold? (b) What is the qualitative effect of the action in (a) on T^{leaf} and the infrared radiation emitted by the leaf? (c) What is the essentially immediate effect on R_{wv}^{total} and J_{wv} of increasing the wind velocity fourfold? (d) What is the qualitative effect of the action in (c) on J_H^C and T^{leaf} ?

7.7 Let us suppose that the net radiation is $0.40 \text{ cal/cm}^2\text{-min}$ for some sunlit leaf with an unstirred layer 1.0 mm thick on each side. Let the leaf temperature be 32° , the air temperature be 30° , D_{wv}^a be $0.26 \text{ cm}^2/\text{sec}$, $D_{\text{CO}_2}^a$ be $0.17 \text{ cm}^2/\text{sec}$, R_{wv}^{ias} be 0.3 sec/cm , and $R_{\text{CO}_2}^{\text{total}}$ be 15 sec/cm . (a) What is J_H^C ? (b) What must J_{wv} be if there is to be no net gain or loss of energy by the leaf? (c) If the relative humidity is 35% in the surrounding air, what are R_{wv}^{total} and R_{wv}^{st} ? Assume that transpiration occurs through stomates on only one side of the leaf, and let c_{wv}^m be $1.86 \mu\text{moles/cm}^3$. (d) Suppose that the leaf begins to wilt and the stomates close to 20% of their former area. What are R_{wv}^{total} and $R_{\text{CO}_2}^{\text{total}}$ then? (e) Neglecting effects caused by changes in leaf temperature, how are the rates of transpiration and photosynthesis affected by the stomatal closure in (d)?

References

- Aylor, D. E., J.-Y. Parlange, and A. D. Krikorian. 1973. Stomatal mechanics. *American Journal of Botany* **60**: 163-171.
- Cowan, I. R., and F. L. Milthorpe. 1968. Plant factors influencing the water status of plant tissues, in *Water Deficits and Plant Structure*, Vol. I, T. T. Kozlowski, ed., Academic Press, New York, pp. 137-193.
- El-Sharkawy, M., and J. Hesketh. 1965. Photosynthesis among species in relation to characteristics of leaf anatomy and CO_2 diffusion resistances. *Crop Science* **5**: 517-521.
- Esau, K. 1965. *Plant Anatomy*, 2nd ed., Wiley, New York.
- Evans, L. T. 1963. *Environmental Control of Plant Growth*, Academic Press, New York.
- Fischer, R. A. 1972. Aspects of potassium accumulation by stomata of *Vicia faba*. *Australian Journal of Biological Sciences* **25**: 1107-1123.
- Forster, R. E., J. T. Edsall, A. B. Otis, and F. J. W. Roughton (eds.). 1969. CO_2 : *Chemical, Biochemical, and Physiological Aspects*, National Aeronautics and Space Administration, Washington, D.C.
- Gates, D. M. 1965. Energy, plants, and ecology. *Ecology* **46**: 1-13.
- Gates, D. M. 1968. Transpiration and leaf temperature. *Annual Review of Plant Physiology* **19**: 211-238.
- Gates, D. M. 1970. Physical and physiological properties of plants, in *Remote Sensing*, National Academy of Sciences, Washington, D.C., pp. 224-252.
- Gates, D. M., and L. E. Papian. 1971. *Atlas of Energy Budgets of Plant Leaves*, Academic Press, New York.

- Geiger, R. 1966. *The Climate near the Ground*, Harvard University Press, Cambridge.
- Hatch, M. D., and C. R. Slack. 1970. Photosynthetic CO₂-fixation pathways. *Annual Review of Plant Physiology* **21**: 141-162.
- Hatch, M. D., C. B. Osmond, and R. O. Slatyer (eds.). 1971. *Photosynthesis and Photorespiration*. Interscience (Wiley), New York.
- Heath, O. V. S. 1969. *The Physiological Aspects of Photosynthesis*, Stanford University Press, Stanford.
- Idso, S. B., D. G. Baker, and D. M. Gates. 1966. The energy environment of plants. *Advances in Agronomy* **18**: 171-218.
- Kramer, P. J. 1969. *Plant and Soil Water Relationships*, McGraw-Hill, New York.
- Kreith, F. 1965. *Principles of Heat Transfer*, 2nd ed., International Textbook Co., Scranton (Pa.).
- Lake, J. V. 1967. Respiration of leaves during photosynthesis. *Australian Journal of Biological Sciences* **20**: 487-499.
- Levitt, J. 1972. *Responses of Plants to Environmental Stresses*, Academic Press, New York.
- Linacre, E. T. 1972. Leaf temperature, diffusive resistances, and transpiration. *Agricultural Meteorology* **10**: 365-382.
- Lommen, P. W., C. R. Schwintzer, C. S. Yocum, and D. M. Gates. 1971. A model describing photosynthesis in terms of gas diffusion and enzyme kinetics. *Planta* **98**: 195-220.
- Maren, T. H. 1967. Carbonic anhydrase: Chemistry, physiology, and inhibition. *Physiological Reviews* **47**: 595-781.
- Meidner, M., and T. A. Mansfield. 1968. *Physiology of Stomata*, McGraw-Hill, New York.
- Munn, R. E. 1966. *Descriptive Micrometeorology*, Academic Press, New York.
- Raschke, K. 1960. Heat transfer between the plant and the environment. *Annual Review of Plant Physiology* **11**: 111-126.
- Robinson, N. (ed.). 1966. *Solar Radiation*, Elsevier, Amsterdam.
- Schwintzer, C. R. 1971. Energy budgets and temperatures of nyctinastic leaves on freezing nights. *Plant Physiology* **48**: 203-207.
- Šetlik, I. (ed.). 1970. *Prediction and Measurement of Photosynthetic Productivity*, Pudoc, Centre for Agricultural Publishing and Documentation, Wageningen (the Netherlands).
- Tolbert, N. E. 1971. Microbodies—peroxisomes and glyoxysomes. *Annual Review of Plant Physiology* **22**: 45-74.
- van Wijk, W. R. (ed.). 1966. *Physics of Plant Environment*, 2nd. ed., North-Holland, Amsterdam.
- Waggoner, P. E. 1969. Predicting the effect upon net photosynthesis of changes in leaf metabolism and physics. *Crop Science* **9**: 315-321.
- Woolley, J. T. 1971. Reflectance and transmittance of light by leaves. *Plant Physiology* **47**: 656-662.
- Zelitch, I. 1971. *Photosynthesis, Photorespiration, and Plant Productivity*, Academic Press, New York.

PLANTS

In the previous chapter we analyzed gas and energy fluxes for a single leaf. We repeatedly used Fick's first law in the following form: flux equals concentration drop divided by resistance. Such an approach can be extended to discuss quantitatively some of the physiological aspects of an entire plant community. We will first describe certain fluxes in the air above the plants. Although the fluxes of water vapor and carbon dioxide in the air above the vegetation resemble diffusion, inasmuch as net migration of such gases is toward regions of lower concentration, we are not dealing with the random thermal motion of molecules. We must consider instead the random motion of relatively large packets of air in the turbulent region above the leaf canopy.

Our next task will be to discuss concentrations and fluxes *within* a plant community. When we begin to analyze the water vapor and carbon dioxide fluxes from the soil up to the tops of plants, we are immediately confronted by the great structural diversity between different types of vegetation. Each plant community has its own unique patterns for the spatial variation of the concentrations of water vapor and carbon dioxide. The many layers of leaves and the changing illumination also greatly complicate the analysis. In fact, even approximate descriptions of the gaseous fluxes within carefully selected plant communities involve complex calculations based on models incorporating numerous simplifying assumptions. To illustrate how carbon dioxide and water vapor concentrations and fluxes within a plant community can be handled in practical calculations, we will consider a cornfield as a specific example in this chapter.

The water transpired by plants comes from the soil. Soil also enters into the CO_2 fluxes and even the energy balance for vegetation. Therefore, after we consider gaseous fluxes within a plant community, we will examine the

thermal and hydraulic conductivity properties of soil. Water in the soil is removed from larger pores before smaller ones. Such removal decreases the soil conductivity for subsequent water movement, and a greater drop in water potential from the bulk soil up to a root is therefore necessary for a given water flux.

Our final topic will be the flow of water in a continuous stream from the soil, to the root, into the root xylem, up to the leaves, and eventually out through the stomata into the atmosphere. As a useful first approximation, we can use the negative gradient of the water potential to represent the driving force for the flux across any segment where water moves as a liquid. We usually replace $-\partial\psi/\partial x$ by $\Delta\psi/\Delta x$. The greater the resistance—or, alternatively, the lower the conductivity—the larger is the $\Delta\psi$ required to maintain a given water flux across a particular component. However, $\Delta\psi$ does not always represent the driving force on water. Also, we must be prepared to recognize interactions between water movement in the xylem and in the other major transport system in plants, the phloem.

Gaseous fluxes above the leaf canopy

In the previous chapter we considered the fluxes of H_2O and CO_2 for individual leaves. We assumed that outside the unstirred air layer on each side of a leaf there is a turbulent region where both water vapor and CO_2 have uniform concentrations. Actually, gradients in both CO_2 and H_2O exist within this turbulent region around plants. We will find that the ambient wind velocity is not constant, but varies with distance from the vegetation.

Wind velocity. Because of frictional drag between the air and a leaf, the air immediately adjacent to the leaf surface is stationary (Fig. 8.1). As we move away from the leaf, a laminar flow of air tends to occur parallel to the leaf surface, its velocity increasing with distance. When we reach a certain distance (δ^a) from the surface, there is a transition from the laminar flow parallel to the leaf to a turbulent flow with eddying motion. The wind velocity increases as we move still further away from the leaf (Fig. 8.1), often increasing logarithmically for a few hundred meters above the leaf canopy. (The entire turbulent region generally extends 0.5 to 1 km above the earth's surface, at which point there is a region of more or less laminar flow in the direction of the prevailing wind.) Since the wind velocity increases in the turbulent region above vegetation, there is some ambiguity in deciding where to measure the ambient wind velocity in order to calculate unstirred layer thicknesses for leaves (cf. Eq. 7.3). Fortunately, the wind velocity

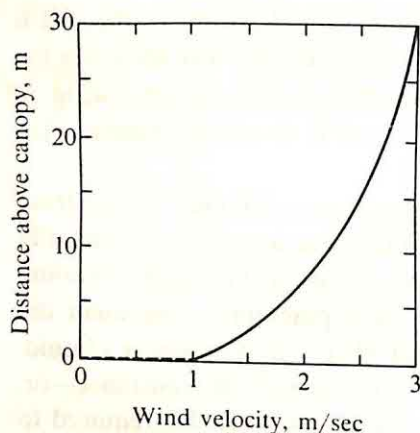


Figure 8.1

Illustration of how wind velocity might increase with distance above a leaf at the top of a canopy. At the leaf surface v^{wind} is zero, while at a distance, δ^a (of the order of mm), it is 1.0 m/sec. At 50 cm into the turbulent air v^{wind} only increases to 1.1 m/sec, while at 30 m above the canopy it triples.

generally does not change very much until we are at least 1 m above the foliage (cf. Fig. 8.1). Thus, wind velocity measured, say, 50 cm above the vegetation is usually accurate enough for most unstirred layer calculations on exposed leaves at the top of the canopy.

Plants exert a frictional drag on the circulating atmosphere and thereby modify the local wind patterns. Since the nature of the vegetation affects the frictional resistance to the wind, it helps determine the wind velocity near the leaves, which in turn sets the average thickness of their unstirred air layers ($\delta^a_{(\text{cm})} = 0.4 \sqrt{l^{\text{leaf}}_{(\text{cm})}/v^{\text{wind}}_{(\text{cm/sec})}}$, Eq. 7.3). The frictional interaction between trees and wind is quite different from that of a flexible crop such as wheat. This leads to a different pattern of wind velocities in the turbulent air (Fig. 8.1) above different types of vegetation (see Cowan; Evans, and Oliver).

Fluxes. A transpiring and photosynthesizing plant community can have a net vertical flux of CO_2 (J_{CO_2}) toward it and a net flux of water vapor (J_{wv}) away from it into the turbulent air above the canopy. In contrast to our use in the previous chapter of leaf area, these fluxes are expressed per unit area of the ground or, equivalently, per unit area of the (horizontal) leaf canopy. Each of the fluxes is related to the appropriate gradient. The vertical flux of water vapor, for example, depends on the rate of change of water vapor concentration in the turbulent air ($c_{\text{wv}}^{\text{ta}}$) with respect to distance (z) above the vegetation:

$$J_{\text{wv}} = -K_{\text{wv}} \frac{\partial c_{\text{wv}}^{\text{ta}}}{\partial z} \quad (8.1)$$

In Equation 8.1 we are again employing the relation: flux equals a

proportionality coefficient times a force, where the force here is the negative gradient of water vapor concentration. Since J_{wv} can be expressed in moles/cm²-sec and $\partial c_{wv}^{ta}/\partial z$ in moles/cm⁴, the coefficient K_{wv} in Equation 8.1 can have units of cm²/sec, the same as for diffusion coefficients. In fact, Equation 8.1 resembles Fick's first law ($J_j = -D_j \partial c_j/\partial x$, Eqs. 1.1 and 7.1) except for one very important distinction: it does not reflect the random thermal motion of water vapor molecules, but the irregular swirling motion of packets, or eddies of air in the turbulent region. This makes the coefficients much larger than for the molecular case.

Before further considering the random eddying motion of air packets, let us write down a general expression for the CO₂ flux in the turbulent air above the canopy. By analogy with Equation 8.1, we can represent the flux of carbon dioxide as

$$J_{CO_2} = -K_{CO_2} \frac{\partial c_{CO_2}^{ta}}{\partial z} \quad (8.2)$$

where K_{CO_2} is the "air packet" ("eddy") diffusion coefficient for carbon dioxide. (K_j is also referred to as a transfer coefficient, an exchange coefficient, or a diffusivity coefficient.) Similarly, the vertical flux of oxygen in the turbulent air (J_{O_2}) could be equated to $-K_{O_2} \partial c_{O_2}^{ta}/\partial z$. We will let the positive direction for z be increasing altitude; consequently, the positive direction for a net flux is from the leaf canopy upward into the turbulent air.

Eddy diffusion coefficients. The eddy diffusion coefficients, K_{wv} and K_{CO_2} , unlike the ordinary diffusion coefficients, D_{wv} and D_{CO_2} , have the *same* value in a given situation. A small packet of air moves more or less as a unit, and thus carries with it the H₂O, CO₂, and other molecules which it contains. Although we cannot really assign actual volumes to these eddies—which are constantly changing in size and shape because of shearing effects or coalescence with neighboring packets—they are indeed very large compared with intermolecular distances, and contain enormous numbers of molecules. The random motion of an air packet is caused by random fluctuations in pressure in local regions of the turbulent air. The eddying motions of the air packets promote a mixing, formally like the mixing due to diffusion, and thus lead to such relations as Equations 8.1 and 8.2. Besides their eddying motion, air packets have an average drift velocity represented by that of the local wind. Pressure gradients over large distances cause the winds and the resulting horizontal drift of the air packets.

The actual values for K_{wv} and K_{CO_2} describing the "diffusion" of air packets vary with the wind speed above the plants. Also, the eddy diffusion

coefficients are affected by the rates of change of both the wind velocity and air temperature with altitude. For instance, hot air tends to rise and become replaced by cooler air. Such buoyancy effects, which are encouraged when $\partial T/\partial z$ is steeply negative above the canopy, lead to more rapid mixing and higher values for K_j . Usually, the eddy diffusion coefficients are approximately proportional to the local wind velocity. As the wind increases, there is more opportunity for turbulent mixing of the air, and thus K_j becomes larger. Since the wind velocity varies with height (cf. Fig. 8.1) and K_j also depends on its gradient, we often employ an eddy diffusion coefficient averaged over an appropriate distance in order to describe the vertical fluxes in some region of the turbulent air above plants. Moreover, the wind velocity, its gradient, and the vertical temperature gradient all vary during the day. Consequently, our value for K_j should also be averaged over a suitable time interval, e.g., an hour.

For a moderate wind velocity of 2 m/sec, the eddy diffusion coefficient is generally 500–2000 cm²/sec just above the leaf canopy. Under these conditions, K_j might be about 15,000 cm²/sec at 30 m above the canopy, and in excess of 30,000 cm²/sec at or above 300 m where turbulent mixing is even greater (see van Wijk). By comparison, D_{wv} is 0.25 cm²/sec and D_{CO_2} is 0.16 cm²/sec in air at 20°. Thus, K_j is 10,000 to 100,000 times larger in the turbulent air than are these D_j s. The random motion of air packets is indeed much more efficient than the random thermal motion of molecules in moving H₂O and CO₂.

Since K_j increases rapidly with altitude as we move into turbulent regions with higher wind velocities, the steady-state concentration gradients become less steep with increasing height above the vegetation. For example, suppose that the net vertical flux of water vapor (J_{wv}) from the plants upward into the turbulent air is essentially constant. Since J_{wv} equals $-K_{wv} \partial c_{wv}^{ta}/\partial z$ by Equation 8.1, and K_{wv} increases with altitude, the absolute value of $\partial c_{wv}^{ta}/\partial z$ must become smaller at greater heights above the canopy. K_j may increase, for example, by a factor of ten in the first 30 m above the vegetation, in which case the gradient in water vapor concentration could decrease 10-fold in this interval.

We have noted that diffusion coefficients are inversely proportional to the square root of molecular weights for gases (p. 18), while the K_j s are all the same in a particular case. This equality of the eddy diffusion coefficients, referred to as the *similarity principle*, results from the air packet and the molecules within it moving as a unit. In fact, K_j is often assumed to be the same for the transfer of gases, heat, and momentum. K_j is therefore generally measured for the most convenient quantity in some situation, and

is then assumed to be the same for all others, following the similarity principle (see Evans; Tanner in Kozlowski, Vol. II, and van Wijk).

Resistance of air above the canopy. Similar to our use in the last chapter of resistances for gaseous diffusion, we will identify a resistance to the flow of water vapor in the turbulent air by R_{wv}^{ta} , and that for CO_2 by $R_{\text{CO}_2}^{ta}$. To derive such quantities, we will replace the negative gradient by the difference in concentration of species j (Δc_j^{ta}) across a given distance (Δz) in the turbulent air, i.e., $-\partial c_j^{ta}/\partial z \cong \Delta c_j^{ta}/\Delta z$. By analogy with our previous definition of resistance ($R_j^a = \Delta c_j^a/J_j = \delta^a/D_j$, Eq. 7.2), we can identify resistances from the flux expressions in Equations 8.1 and 8.2. Since K_{wv} has the same value as K_{CO_2} , we obtain the following equalities:

$$\begin{aligned} R_{wv}^{ta} &= \frac{\Delta c_{wv}^{ta}}{J_{wv}} = \frac{\Delta z}{K_{wv}} \\ &= \frac{\Delta z}{K_{\text{CO}_2}} = \frac{\Delta c_{\text{CO}_2}^{ta}}{J_{\text{CO}_2}} = R_{\text{CO}_2}^{ta} \end{aligned} \quad (8.3)$$

As is the case for the analogous relations in Chapter 7 (e.g., Eqs. 7.2, 7.4, and 7.5), Equation 8.3 describes the steady state condition. Equation 8.3 indicates that R_{wv}^{ta} has the same value as $R_{\text{CO}_2}^{ta}$, as we would indeed expect based on the random motions of whole packets of air.

Let us now estimate the resistance of the turbulent air immediately above a leaf canopy. For simplicity, we will let K_j have an average value of 10,000 cm²/sec for the first 30 m above the plants, a typical value in a moderate wind during the daytime. By Equation 8.3, R_{wv}^{ta} and $R_{\text{CO}_2}^{ta}$ are then (3000 cm)/(10,000 cm²/sec), or 0.3 sec/cm. Measured values for these resistances generally range from 0.2 to 0.4 sec/cm for moderate wind velocities, as do computer analyses of R_j^{ta} using models incorporating the variation of K_j with altitude. Wind velocities, and therefore K_j , tend to be lower at night so that R_j^{ta} is then somewhat higher than during the daytime (see Monteith in Evans).

Transpiration and photosynthesis. As mentioned, we will express the flux of water vapor above plants per unit area of the ground, or, equivalently, per unit area of the canopy. For many agricultural as well as ecological considerations, such a measure of the average transpiration of the whole plant community is far more useful than the water vapor flux of individual leaves. Environmental measurements in the turbulent air above vegetation can thus be a very important approach for measuring the overall rates of transpiration and photosynthesis, especially if the extent of similar plants is fairly large, as might occur for a wheat field. Moreover, such measurements

can generally be made without disturbing the plants or their leaves. On this large scale, however, we unavoidably lose sight of certain factors, such as the effect of stomatal opening or leaf size on the gas fluxes. Also, the turbulent air above the canopy is highly influenced by the terrain as well as by the ground cover, so that we must reckon with other factors not involved in our study of leaves. For example, the gradients in water vapor or CO_2 and K_j depend on whether we are at the edge or the center of a field, whether and what types of trees are present, and whether the region is flat or hilly.

For simplicity, we are considering a one-dimensional situation where the net fluxes of water vapor and CO_2 occur only in the vertical direction above the canopy, as would be the case near the center of a large uniform plant community. Just as our assumption of a boundary layer of uniform thickness breaks down at the leading and trailing edges of a leaf, we must also consider air packets transferring H_2O and CO_2 horizontally in and out at the sides of certain areas of vegetation. Such net horizontal transfer of various gases is referred to as *advection*. Instead of using Equations 8.1 through 8.3 to analyze net gas fluxes, we may have to use much more cumbersome three-dimensional equations in order to handle advection for small fields or individual plants.

For a representative sunlit mesophytic leaf, the value of J_{wv}/J_{CO_2} might be about 370, while this ratio can be 100 for a photosynthetically efficient species such as corn (p. 342). The moles of water lost per mole of CO_2 fixed is often considerably higher for an entire plant community than for a favorably oriented leaf. In particular, much of the water vapor constituting J_{wv} in the turbulent air comes from the soil and from leaves that are not well-illuminated and which therefore contribute little to net photosynthesis. A plant community uses not only CO_2 from the turbulent air above the canopy, but also CO_2 evolved by soil microorganisms, by root cells, and by respiration in leaves which do not receive much sunlight and so are below the light compensation point. Both of these conditions tend to raise J_{wv}/J_{CO_2} above the values for an exposed leaf. Although the absolute value of J_{wv}/J_{CO_2} above a leaf canopy depends on the ambient relative humidity and the physiological status of the plants, it is generally between 300 and 2000 when averaged over a day in the growing season. Moreover, mainly because J_{CO_2} for C-4 plants is often about twice as large as for photorespiring C-3 plants, the absolute value of J_{wv}/J_{CO_2} is lower and daily growth is greater for the nonphotorespiring plants (see Kozlowski, Vol. II; Kramer, and Zelitch).

J_{CO_2} and $c_{\text{CO}_2}^{\text{ta}}$. We will use representative values of J_{CO_2} in order to

calculate the concentration drops of CO_2 which might be expected over a certain vertical distance in the turbulent atmosphere. When there is net photosynthesis, the net flux of CO_2 is directed from the turbulent air down into the canopy. J_{CO_2} above the vegetation is then negative by our sign convention, which means that $c_{\text{CO}_2}^{\text{ta}}$ increases as we go vertically upward ($J_{\text{CO}_2} = -K_{\text{CO}_2} \partial c_{\text{CO}_2}^{\text{ta}} / \partial z$, Eq. 8.2). But this is just as we would expect if CO_2 is to be transferred downward toward the plants by the random motion of the eddies in the turbulent air.

A commonly observed value for J_{CO_2} above the plant canopy is -2 nmoles/cm²-sec at midday. (For comparison, the flux of CO_2 into an exposed leaf of a mesophyte at a moderate light intensity might be 1.1 nmoles/cm²-sec, p. 340.) Using Equation 8.3 ($R_{\text{CO}_2}^{\text{ta}} = \Delta c_{\text{CO}_2}^{\text{ta}} / J_{\text{CO}_2}$) and a value of 0.3 sec/cm for the resistance of the lower 30 m of the turbulent air ($R_{\text{CO}_2}^{\text{ta}}$), we can calculate that $\Delta c_{\text{CO}_2}^{\text{ta}}$ might be $(-2 \text{ nmoles/cm}^2\text{-sec})(0.3 \text{ sec/cm})$, or $-0.6 \text{ nmole of CO}_2/\text{cm}^3$.^{*} Employing a conversion factor from Table 7.2, this $\Delta c_{\text{CO}_2}^{\text{ta}}$ corresponds to a drop of $(0.6)(24.4)$, or 15 ppm CO_2 by volume at 20° and a pressure of 1 bar. Thus, CO_2 , which generally has a concentration near 320 ppm well into the turbulent air, e.g., 30 m above vegetation, could be at 320 - 15, or 305 ppm just above the canopy (see Loomis *et al.*; Partridge; Šetlik; San Pietro *et al.*, and Zelitch).

For a rapidly photosynthesizing corn crop at noon, J_{CO_2} can be -6 nmoles/cm²-sec. For an $R_{\text{CO}_2}^{\text{ta}}$ of 0.3 sec/cm, $\Delta c_{\text{CO}_2}^{\text{ta}}$ would then be $(-6)(0.3)(24.4)$, or about -40 ppm by Equation 8.3. Thus, the CO_2 concentration at the top of the canopy can be 40 ppm lower, e.g., 280 ppm, than in the turbulent air tens of meters above the corn plants. In fact, measurement of $c_{\text{CO}_2}^{\text{ta}}$ at the canopy level can indicate the net rate of photosynthesis by the plants. Because of nocturnal respiration without photosynthesis, vegetation acts like a source of CO_2 at night. Thus, the concentration of CO_2 just above the canopy at night is usually a few ppm greater than it is higher up in the turbulent air, as we will indicate below.

$J_{\text{H}_2\text{O}}$ and $c_{\text{H}_2\text{O}}^{\text{ta}}$. The flux of water vapor just above the canopy, which represents transpiration from the leaves plus evaporation from the soil, is often referred to as *evapotranspiration*. For fairly dense vegetation such evapotranspiration is appreciable, amounting to 60-90% of the flux of water vapor from a layer of open water at the ambient air temperature. The daily evapotranspiration from a forest often corresponds to a layer of water 0.3-0.5 cm thick, which averages out to 0.2-0.3 $\mu\text{mole/cm}^2\text{-sec}$ (see Kozlow-

^{*} By analogy with Ohm's law ($\Delta E = IR$), in this chapter we will use the form $\Delta c_j^{\text{ta}} = J_j R_j^{\text{ta}}$ for our calculations of concentration drops.

ski, Vol. II; Kramer; and Slatyer). At noon on a sunny day with a moderate wind J_{wv} above a leaf canopy can be $0.7 \mu\text{mole of water/cm}^2\text{-sec}$. By Equation 8.3 ($\Delta c_{wv}^{ta} = J_{wv} R_{wv}^{ta}$) and employing a value of 0.3 sec/cm for R_{wv}^{ta} , we can calculate that the concentration drop of water vapor in the turbulent air would be $(0.7 \mu\text{mole/cm}^2\text{-sec})(0.3 \text{ sec/cm})$, or $0.2 \mu\text{mole/cm}^3$. We indicated in Chapter 7 that the turbulent air immediately outside the unstirred layers adjacent to a leaf contains $0.48 \mu\text{mole of water/cm}^3$ when it is at 20° and 50% relative humidity. Our calculation indicates that c_{wv}^{ta} could drop by $0.2 \mu\text{mole/cm}^3$, or from 0.48 to about $0.3 \mu\text{mole/cm}^3$ (near 30% relative humidity at 20°) as we move 30 m upward into the turbulent air above the canopy. Such a marked decrease in absolute and relative humidity is indeed often observed in the turbulent air above vegetation.

Let us next analyze the "steaming" of leaves, or even of a forest, which occurs when the sun breaks through the clouds following a rainstorm. We will assume that the sun warms up the leaves at the top of the canopy to 25° and that the concentration of water vapor in the cell wall pores of their mesophyll cells is then $1.27 \mu\text{moles/cm}^3$ (cf. p. 320). Suppose that the air just outside the unstirred layer adjacent to a leaf is at 22° and has a high relative humidity of 95% just after the rainstorm; c_{wv}^{ta} is then $1.03 \mu\text{moles/cm}^3$ ($c_{wv}^* = 1.08 \mu\text{moles/cm}^3$ at 22°). Hence, water vapor will diffuse from the leaf, across the unstirred layer, into the turbulent air. Now let us suppose that the air at a greater distance from the leaf is somewhat cooler, e.g., 20° a few cm from the leaf. At 20° c_{wv}^* is $0.96 \mu\text{mole/cm}^3$. Thus, as the air with $1.03 \mu\text{moles of water/cm}^3$ moves away from the unstirred layer adjacent to the leaf in an eddy, or air parcel, it will be cooled and some of its water vapor will condense, since c_{wv}^{ta} cannot exceed c_{wv}^* for the local air temperature. This condensation leads to a fog, or "steam" moving away from the plants into the surrounding cooler turbulent air, a phenomenon which is commonly observed after a rainstorm or at sunrise following a night with a high relative humidity.

Gas fluxes within plant communities

The pattern of concentrations and fluxes obviously depends on the particular plant community being considered. We will not attempt to examine all types of vegetation, instead we will focus on a cornfield (see Lemon *et al.*). The same general principles apply to other fairly uniform plant communities, while isolated plants provide special difficulty for analysis, since the gas concentrations and fluxes then vary in three dimensions.

Eddy diffusion coefficient and resistance. Let us begin by considering how the eddy diffusion coefficient might vary within a plant community. Near the ground there is an unstirred boundary layer which can be rather thick, since the air there is generally quite still. In fact, K_j often averages $0.5 \text{ cm}^2/\text{sec}$ in the first cm above the ground, a value which is only 2 to 3 times larger than the diffusion coefficients of water vapor and CO_2 in air. As we move up to the top of the canopy, the eddy diffusion coefficient increases, often more or less logarithmically with altitude in the upper part of many plant communities. It may reach a value of $2000 \text{ cm}^2/\text{sec}$ at the top of a canopy in a moderate wind (Fig. 8.2). Since K_j is approximately proportional to wind velocity, v^{wind} within the plant community varies in a manner similar to the variation described for the eddy diffusion coefficient (Fig. 8.2). For instance, the wind velocity about 0.2 m above the ground might be 0.1 m/sec, increasing to 2 m/sec at the top of the canopy (see Millington and Peters).

Two points concerning K_j within the plant community deserve special emphasis. First, transfer of gaseous substances within the vegetation takes place by the random motion of relatively large parcels or eddies of air, just like in the turbulent region above the canopy. Second, because of frictional drag with the many leaves, branches, and other plant parts, the eddy diffusion coefficient within the vegetation is considerably less than in the air above the canopy.

We will now estimate the resistance of the turbulent air from the ground to the top of a corn crop 2 m in height (Fig. 8.2). To illustrate the relative contributions of various air layers, we will let K_j average $0.5 \text{ cm}^2/\text{sec}$ for the first 1 cm, $10 \text{ cm}^2/\text{sec}$ for the next 9 cm, $200 \text{ cm}^2/\text{sec}$ for the next 90 cm, and $1000 \text{ cm}^2/\text{sec}$ for the last 100 cm, consistent with the plot of K_j versus

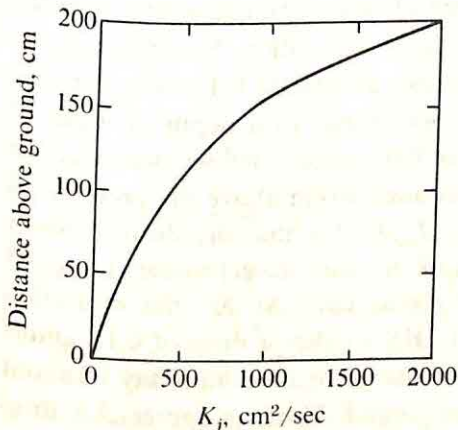


Figure 8.2

Idealized representation of the variation in the eddy diffusion coefficient within a uniform corn crop 2 m in height. The wind velocity is 2 m/sec at the top of the canopy.

Table 8.1

Summary of eddy diffusion coefficients, resistances, and both water vapor and carbon dioxide fluxes averaged over various intervals within a 2 m high corn crop on a sunny day. Just above the canopy, K_j is 2000 cm²/sec, J_{wv} is 0.7 μ mole/cm²-sec, and J_{CO_2} is -6 nmoles/cm²-sec.

Height above ground (cm)	avg K_j (cm ² /sec)	R_j^{ta} (sec/cm)	avg J_{wv} (μ mole/cm ² -sec)	avg J_{CO_2} (nmole/cm ² -sec)
0 to 1	0.5	2.0	0.05	0.2
1 to 10	10	0.9	0.05	0.2
10 to 100	200	0.5	0.1	0.3
100 to 200	1000	0.1	0.4	-3

height in Figure 8.2 (also see Table 8.1). Using Equation 8.3 ($R_{wv}^{ta} = \Delta z/K_{wv} = R_{CO_2}^{ta}$) to estimate the resistance of each of these four air layers in series, we can calculate that the overall resistance will be (1 cm)/(0.5 cm²/sec) + (9 cm)/(10 cm²/sec) + (90 cm)/(200 cm²/sec) + (100 cm)/(1000 cm²/sec), which is 2.0 + 0.9 + 0.5 + 0.1, or 3.5 sec/cm (summarized in Table 8.1). (In a sense, we are performing a numerical integration in order to determine the resistance.) Computer analyses using models describing the turbulent air within such a crop also indicate that the resistance is generally 3-4 sec/cm. Most of the resistance within a plant community is generally due to the relatively still air next to the ground. For instance, just over half (2.0 sec/cm out of 3.5 sec/cm) of the resistance for the 2 m pathway is provided by the lowest 1 cm, while the entire upper half of the corn crop accounts for a resistance of only 0.1 sec/cm (cf. Table 8.1).

Water vapor. A considerable amount of water can evaporate from the soil and move by air packets up through the vegetation. For instance, J_{wv} from a moist, intermittently illuminated soil can be 0.02-0.1 μ mole/cm²-sec. (By comparison, 0.05 μ mole/cm²-sec corresponds to a depth of water of 0.8 mm/day or 28 cm/year.) If a flow of 0.05 μ mole/cm²-sec occurs across a resistance of 2.9 sec/cm to reach a distance 10 cm above the ground, we estimate, using Equation 8.3 ($\Delta c_{wv}^{ta} = J_{wv} R_{wv}^{ta}$), that the drop in water vapor concentration from the ground to this level would be (0.05 μ mole/cm²-sec) (2.9 sec/cm), or 0.15 μ mole/cm³. At 20° the saturation concentration is 0.96 μ mole/cm³ (App. II), so that a drop of 0.15 μ mole water vapor/cm³ corresponds to a decrease in relative humidity of about 15% at 20° across this 10 cm above the ground. Thus, an appreciable drop

in water vapor concentration can occur across the relatively still air near a moist soil under a canopy (Fig. 8.3).

As we move from the ground up through a corn crop, J_{wv} increases because of the addition of water vapor due to transpiration by the leaves. On a sunny day the water vapor flux in $\mu\text{moles}/\text{cm}^2\text{-sec}$ might be 0.1 at 50 cm, 0.2 at 100 cm, 0.4 at 150 cm, and 0.7 at 200 cm, the top of the canopy (on a cloudy humid day J_{wv} for a corn crop might be only 0.1 $\mu\text{mole}/\text{cm}^2\text{-sec}$ at the top of the canopy). If J_{wv} averages 0.1 $\mu\text{mole}/\text{cm}^2\text{-sec}$ from 10 to 100 cm above the ground, where the resistance is 0.5 sec/cm (Table 8.1), then by Equation 8.3 $\Delta c_{wv}^{\text{ta}}$ for this part of the pathway is $(0.1 \mu\text{mole}/\text{cm}^2\text{-sec})(0.5 \text{ sec}/\text{cm})$, or 0.05 $\mu\text{mole}/\text{cm}^3$. For the next 100 cm the resistance is 0.1 sec/cm, so that for an average J_{wv} of 0.4 $\mu\text{mole}/\text{cm}^2\text{-sec}$ (Table 8.1) the decrease in water vapor concentration would be $(0.4)(0.1)$, or 0.04 $\mu\text{mole}/\text{cm}^3$. Thus, $\Delta c_{wv}^{\text{ta}}$ in $\mu\text{moles}/\text{cm}^3$ is 0.15 over the 10 cm just above the ground, 0.05 from 10 to 100 cm, and 0.04 from 100 to 200 cm, or 0.24 overall (cf. Fig. 8.3). If the turbulent air at the top of the canopy is at 20° and 50% relative humidity, it would contain 0.48 μmole of water/ cm^3 . The air near the soil would then contain approximately $0.48 + 0.24$, or 0.72 $\mu\text{mole H}_2\text{O}/\text{cm}^3$, which corresponds to 75% relative humidity at 20° ($c_{wv}^* = 0.96 \mu\text{mole}/\text{cm}^3$ at 20°, App. II). In summary, we note that (1) air close to the soil under a (fairly dense) leaf canopy can have an appreciable relative humidity (2) c_{wv}^{ta} continuously decreases as we move upward from the ground (3) most of the overall water vapor drop occurs near the ground, and (4) most of the water vapor actually comes from the upper half of the corn crop in the present example.

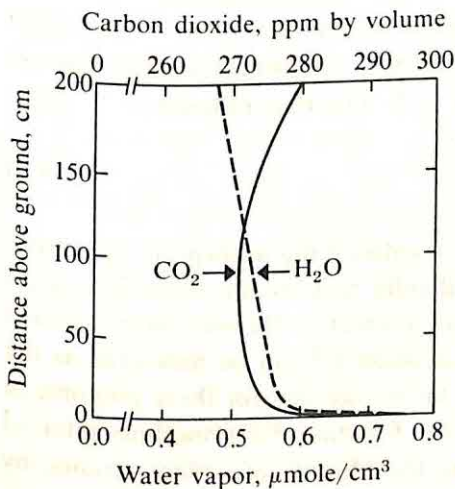


Figure 8.3

Possible variation of water vapor and carbon dioxide concentrations within a 2 m high corn crop at noon on a sunny day. At the top of the canopy v^{wind} is 2 m/sec, while in the turbulent air 30 m above the vegetation c_{wv}^{ta} is 0.3 $\mu\text{mole}/\text{cm}^3$ and $c_{\text{CO}_2}^{\text{ta}}$ corresponds to 320 ppm by volume. (See Lemon *et al.* for actual values under field conditions.)

Illumination. Before discussing J_{CO_2} within a plant community, we should consider how the amount of light varies down through the various layers of vegetation, since the illumination at each level determines the rate of photosynthesis there. The net rate of CO_2 fixation is light-saturated near 40,000 lux for many leaves and it decreases to zero at the light compensation point. A comprehensive formulation including effects of leaf angle, sun elevation in the sky, the finite width of the sun's disc, changes in spectral distribution of the light at various levels within the plant community, multiple reflections of the light from leaves and other surfaces, as well as clumping versus uniform arrangement of leaves would lead to a hopeless complication of the algebra. Instead, we will assume that the decrease in illumination is due to light absorption by the foliage in a manner analogous to Beer's law, $\ln(I/I_0) = -k_\lambda cb$, (Eq. 4.14). This approximation is particularly useful when there is a random distribution of leaves horizontally, as can occur in many moderately dense plant communities.

As we move down into the vegetation, the illumination decreases more or less exponentially with the amount of absorbing material encountered. We will let F be the average cumulative total leaf area per unit ground area as we move down through the plant community. The dimensionless parameter F uses the area of only one side of a leaf, thus it is expressed on the same basis as our fluxes. F is zero at the top of the canopy, and takes on its maximum value at ground level, a value generally referred to as the *leaf area index*. If the leaves in a particular plant community were horizontal, the leaf area index would equal the average number of leaves above the ground.

We will represent the photon flux in the visible range (approximately $0.4\text{--}0.7\ \mu\text{m}$) incident on the leaf canopy by I_0 . Primarily because of absorption by photosynthetic pigments, light is attenuated as it moves down through the plant community. At any level in the vegetation, the photon flux in the visible region (I) is related to I_0 and F as follows:

$$\ln \frac{I}{I_0} = -kF \quad (8.4)$$

where k is a dimensionless parameter describing the absorption properties of a particular type of foliage; we will refer to k as the *foliar absorption coefficient*. Since we are ignoring changes in spectral distribution at different levels in the vegetation, I_0 and I in Equation 8.4 can be measured as the flux of photons from 0.4 to $0.7\ \mu\text{m}$, as an energy flux for these photons, or even in lux, a unit referring to visible light. Equation 8.4, sometimes referred to as the Bouguer-Lambert law, was introduced into plant studies by

M. Monsi and T. Saeki in 1953 (see Eastin *et al.*; Saeki in Evans; Šetlik, and Zelitch).

Values of foliar absorption coefficients. The foliar absorption coefficient k has values between 0.3 and 1.2 for most leaf canopies. Light penetrates the vertically oriented blades of grasses rather easily; in such cases k often has a low value near 0.4. At what cumulative leaf area per ground area would the incident visible light be reduced by 95% for grasses with a foliar absorption coefficient of 0.4? By Equation 8.4, F would be $[\ln (0.05/1.00)]/(-0.4)$, or $\ln 20/(0.4)$, which is 7.5. Thus, when the average leaf area index is 7.5 for such grasses, 5% of the sunlight would reach the soil surface. In order for 95% of the visible light to be absorbed for a leaf area index of 3, k must be $\ln 20/(3)$, or 1.0, by Equation 8.4. Such a relatively high foliar absorption coefficient applies to horizontal leaves with at least 0.05 mg chlorophyll/cm².

When the sun is overhead vertical leaves absorb less sunlight and reflect more down into the vegetation per unit leaf area than do horizontal leaves. This accounts for the low values of k for grasses, since their leaves are generally rather erect. Moreover, leaves tend to be vertical near the top of certain plants, e.g., sugar beet, becoming on the average more horizontal toward the ground. This orientation reduces the foliar absorption coefficient of the upper leaves, therefore more of the light incident on the plants is available for the lower leaves. In fact, optimal light utilization generally occurs when the incident light is distributed as uniformly as possible over the leaves, since the fraction of leaves exposed to illuminations above light saturation or below light compensation is then usually minimized (see Moss and Musgrave as well as Yoshida). Our arguments about the effect of leaf orientation on k presuppose that essentially all of the light is incident on the top of the canopy. When much illumination comes in from the sides, as for an isolated tree, foliar absorption coefficients determined for vertically incident light should not be used in Equation 8.4.

Light compensation point. Let us now consider the light compensation point for CO₂ fixation by leaves. As we mentioned in the last chapter (p. 339), the light compensation point is generally close to 400 lux for a leaf temperature near 20° and a CO₂ concentration of 300 ppm (c_{CO_2} can be below this level within the plant community, as we will show below). Suppose that a moderate illumination of 20,000 lux occurs on trees whose leaves have a foliar absorption coefficient of 0.8. At what cumulative area of leaves per unit ground area is a light compensation point of 400 lux reached? By Equation 8.4, F is $\ln (400 \text{ lux}/20,000 \text{ lux})/(-0.8)$, or 4.9. Thus, only the upper five "layers" of leaves in a dense forest might be above the light

compensation point for that part of the day when the illumination on the canopy is 20,000 lux. Of course, for a lower illumination on the leaf canopy, more leaves would be below the light compensation point. Occasional sun flecks of high illumination reach the lower parts of the vegetation, which can complicate our analysis of where light compensation occurs.

Not only is photosynthesis less at lower illuminations, the stomates in poorly lit leaves also tend to close, which increases stomatal resistance to the diffusion of CO_2 . Stomatal closure in many leaves actually begins when the illumination drops below 10,000 lux. Leaves which are below the light compensation point for most of the day do not contribute to the net photosynthesis of the plant because of insufficient sunlight penetrating down to them. Such leaves generally lose 30–50% of their dry weight before actually dying and abscising. Following such loss of leaves on the lower branches of trees, the branches themselves die and eventually fall off or are blown off by the wind. Thus, tall trees in a dense forest often have few or no branches over the lower part of their trunks.

CO_2 concentrations and fluxes. In contrast to the concentration of water vapor, which continuously decreases with distance above the ground, the CO_2 concentration generally achieves a minimum somewhere within the plant community on a sunny day (Fig. 8.3). This occurs because both the turbulent air above the canopy and the soil can serve as sources of CO_2 . During the day CO_2 diffuses down one concentration gradient directed from the soil up into the vegetation, and down another directed from the overlying turbulent air into the leaf canopy (see Eastin *et al.*, and Zelitch).

Respiration in root cells and in soil microorganisms can lead to a net upward CO_2 flux from the ground of 0.1 to 0.3 nmole/cm²-sec during the growing season. (An oxygen flux of similar magnitude occurs in the opposite direction.) The actual value of J_{CO_2} from the soil varies diurnally in phase with the soil temperature, which is higher during the day. We have already estimated that $R_{\text{CO}_2}^{\text{ta}}$, which is the same as $R_{\text{CO}_2}^{\text{ta}}$, might be 2.0 sec/cm for the first 1 cm, and 0.9 sec/cm for the next 9 cm above the ground for a corn crop 2 m tall (Table 8.1). Using Equation 8.3 ($\Delta c_{\text{CO}_2}^{\text{ta}} = J_{\text{CO}_2} R_{\text{CO}_2}^{\text{ta}}$), we calculate that for a moderate CO_2 flux of 0.2 nmole/cm²-sec emanating from the soil the decrease in CO_2 concentration across the first cm above the ground is (0.2 nmole/cm²-sec)(2.0 sec/cm), or 0.4 nmole/cm³. By the conversion factor in Table 7.2, this corresponds to a drop of approximately (0.4 nmole/cm³)[24.4 ppm CO_2 /(nmole/cm³)], or 10 ppm CO_2 by volume at 20° and 1 bar pressure. In the next 9 cm, the CO_2 concentration might decrease [(0.9 sec/cm)/(2.0 sec/cm)] (10 ppm), or about 5 ppm. Thus, the CO_2 level may drop 15 ppm from 290 ppm at the soil surface to 275 ppm at 10 cm above the ground (Fig. 8.3).

As we move upward from the ground, the flux of CO_2 initially increases as we encounter leaves which are below the light compensation point and which thus have a net evolution of CO_2 . For instance, J_{CO_2} directed upward may increase from 0.2 nmole/cm²-sec at 10 cm to 0.5 nmole/cm²-sec at 50 cm. As we move even higher and encounter leaves with net photosynthesis, the net flux of CO_2 in the turbulent air decreases, and may become zero at 100 cm above the ground in a 2 m high corn crop with a high photosynthetic rate. Thus, J_{CO_2} may average 0.3 nmole/cm²-sec from 10 to 100 cm above the ground, an interval which has a resistance of 0.5 sec/cm (Table 8.1). This would lead to a $\Delta c_{\text{CO}_2}^{\text{ta}}$ of (0.3)(0.5), or 0.15 nmole/cm³, which corresponds to a decrease of (0.15)(24.4), or nearly 4 ppm of CO_2 . Hence, the CO_2 concentration might reach its lowest value of just over 270 ppm at the midway point in the crop (cf. Fig. 8.3).

At noon on a sunny day, J_{CO_2} down into a cornfield might be 6 nmoles/cm²-sec. Essentially all of the net CO_2 flux from the turbulent air above the canopy is directed into the leaves in the upper half of the corn crop, e.g., J_{CO_2} may become -3 nmoles/cm²-sec at 150 cm above the ground and zero at 100 cm. Thus, the average CO_2 flux in the upper half of the vegetation is about -3 nmoles/cm²-sec, while the resistance is 0.1 sec/cm (Table 8.1). Consequently, $\Delta c_{\text{CO}_2}^{\text{ta}}$ for an actively photosynthesizing cornfield might be (-3 nmoles/cm²-sec)(0.1 sec/cm) [24.4 ppm CO_2 /(nmole/cm³)], or slightly more than a 7 ppm decrease from the top of the canopy to 1 m below (Fig. 8.3). We indicated on p. 373 that $c_{\text{CO}_2}^{\text{ta}}$ at the top of this canopy might be about 280 ppm. The carbon dioxide concentration 100 cm above the ground would then be just over 270 ppm by volume, the same value we estimated by working our way up from the ground.

CO_2 concentrations in the air can vary over quite a wide range for different plant communities. For a cornfield exposed to a low wind velocity (below 0.3 m/sec at the top of the canopy), for a rapidly growing plant community, or for other dense vegetation where the eddy diffusion coefficient may be relatively small, the CO_2 concentration in the turbulent air within the plant stand can drop below 200 ppm during a sunny day. On the other hand, for sparse vegetation, especially on windy or overcast days, $c_{\text{CO}_2}^{\text{ta}}$ generally does not decrease even 1 ppm from the value at the top of the canopy.

CO_2 at night. The CO_2 concentration at night is highest near the ground and continuously decreases as we go upward through the plants into the turbulent air above. J_{CO_2} emanating from the soil might be 0.1 nmole/cm²-sec, while the respiratory flux of CO_2 from the parts of the plant above the ground can be 0.3 nmole/cm²-sec at night. Respiration can be 20% of gross photosynthesis for a rapidly growing plant community, and can in-

crease to over 50% as the community matures. For certain climax communities, plant respiration can become nearly 100% of gross photosynthesis. When considered for a growing season, respiration for an entire plant is generally 30–50% of total photosynthesis.

We will now estimate CO_2 concentration drops which might occur in a 2 m high crop at night. For purposes of calculation, let us assume that J_{CO_2} vertically upward averages 0.1 nmole/cm²-sec over the first 10 cm where R_j^{ta} is 3.0 sec/cm, 0.2 nmole/cm²-sec over the next 90 cm where the resistance is 0.5 sec/cm, and 0.3 nmole/cm²-sec over the final 100 cm where R_j^{ta} may be 0.2 sec/cm at night. By Equation 8.3 ($\Delta c_{\text{CO}_2}^{\text{ta}} = J_{\text{CO}_2} R_{\text{CO}_2}^{\text{ta}}$), the drop in CO_2 concentration from the ground to the top of the canopy is $(0.1)(3.0) + (0.2)(0.5) + (0.3)(0.2)$, or 0.46 nmole/cm³, which corresponds to $(0.46)(24.4)$, or about 10 ppm. The resistance of the first 30 m of turbulent air above the plants might be 0.5 sec/cm at night; we will let the total CO_2 flux from the canopy be 0.4 nmole/cm²-sec. Thus, $\Delta c_{\text{CO}_2}^{\text{ta}}$ for the first 30 m above the canopy could be $(0.4 \text{ nmole/cm}^2\text{-sec})(0.5 \text{ sec/cm})$, or 0.2 nmole/cm³, which is $(0.2)(24.4)$ or 5 ppm CO_2 by volume, using the conversion factor in Table 7.2. If $c_{\text{CO}_2}^{\text{ta}}$ is 320 ppm 30 m up in the turbulent air, the CO_2 concentration at night might be about 325 ppm at the top of the canopy, and close to 335 ppm at the soil surface. Such estimates are in agreement with measurements of CO_2 concentrations at night.

Soil

Soils vary tremendously in their physical properties, such as the size of the individual particles. In a sandy soil many particles are over 1 mm in diameter, while in a fine clay most of the particles are less than 3 μm in diameter. Small soil particles have a much greater surface area per unit weight than do large particles. Sand, for example, can have under 1 m² of surface area/g, while most clays have 100–1000 m² of surface area/g. Most soil minerals are aluminosilicates, having negatively charged surfaces which act like Donnan phases (cf. p. 116) with the mobile cations in the adjacent soil water. Because of their large surface areas per unit weight, clays dominate the ion exchange properties of many soils. The clay montmorillonite, for example, has 800 m² of surface area/g, and can hold nearly 1 milliequivalent of cations/g (see Childs, and Hillel).

The irregularly shaped pores between the numerous particles in the soil contain both air and water (cf. Fig. 8.4). The soil pores, or voids, vary from somewhat under 40% to about 60% of the soil by volume. Thus, a soil whose

pores are completely filled with water will contain 40–60% water by volume. In the vicinity of most roots the soil contains 5–25% water by volume, while the rest of the pore space is filled with air. The pores therefore provide many air-liquid interfaces where surface tension effects can lead to a negative hydrostatic pressure in the soil water. Such a negative P is generally the main contributor to the water potential in the soil, especially as the soil dries out.

Thermal properties. To raise the temperature of 1 g of dry sand by 1° takes about 0.2 cal, with similar values for dry clay or loam. Since the density of soil solids is about 2.6 g/cm^3 and soil is about half pores by volume, the overall density of dry soil is about 1.3 g/cm^3 —actual values generally range from 1.2 g/cm^3 for dry loam, slightly higher for clay, and up to 1.5 g/cm^3 for dry sand. Thus, the volumetric heat capacity of dry soil is about $0.3 \text{ cal/cm}^3\text{-}^\circ$; for a moist soil containing 20% water by volume, it is about $0.5 \text{ cal/cm}^3\text{-}^\circ$ (see van Wijk).

One consequence of the relatively high heat capacity of soil is that a considerable amount of energy can be involved with its temperature changes. For instance, if the soil temperature for the upper 40 cm of a moist soil with a heat capacity of $0.5 \text{ cal/cm}^3\text{-}^\circ$ increases by an average of 2° during the daytime, then $(40 \text{ cm})(2^\circ)(0.5 \text{ cal/cm}^3\text{-}^\circ)$, or 40 cal can be stored per cm^2 of ground. Nearly all the heat stored by the soil during a day is released that night, since the temperature near the top of the soil changes in a diurnal fashion. Generally, the soil temperature at depths below 40 cm changes less than 0.5° during a day or night, although annually it will vary considerably more.

In addition to a substantial volumetric heat capacity, soil also has a relatively high thermal conductivity coefficient, K^{soil} . Heat is therefore readily conducted in a soil, where the heat flux by conduction (J_H^C) is

$$J_H^C = -K^{\text{soil}} \frac{\partial T}{\partial z} \quad (8.5)$$

Heat can of course be conducted in all directions in the soil, instead of only vertically as we will consider here (cf. Eq. 8.5). For Equation 8.5, J_H^C can be expressed in $\text{cal/cm}^2\text{-min}$, the temperature gradient in $^\circ/\text{cm}$, and therefore the soil thermal conductivity coefficient in $(\text{cal/cm}^2\text{-min})/(^\circ/\text{cm})$. The thermal conductivity coefficient of soil depends on its water content; the replacement of soil air (a relatively poor heat conductor) by water will increase K^{soil} . For instance, K^{soil} may be $0.03 (\text{cal/cm}^2\text{-min})/(^\circ/\text{cm})$ for a dry soil and $0.3 (\text{cal/cm}^2\text{-min})/(^\circ/\text{cm})$ for a wet one—by way of comparison, K^{water} is $0.086 (\text{cal/cm}^2\text{-min})/(^\circ/\text{cm})$ and K^{air} is $0.0034 (\text{cal/cm}^2\text{-min})/(^\circ/\text{cm})$ near 20° (see App. II; Rose, and van Wijk).

During the daytime the surface of the ground can be considerably warmer than the underlying layers of the soil— $\partial T/\partial z$ is often about $1^\circ/\text{cm}$ near the surface. Soil exposed to the turbulent air tends to be drier than the underlying layers, hence the thermal conductivity coefficient can be lower near the surface. For the upper part of a fairly moist sandy loam, K^{soil} may be $0.1 \text{ (cal/cm}^2\text{-min)/}(^\circ/\text{cm})$. Using this K^{soil} and a $\partial T/\partial z$ of $1^\circ/\text{cm}$, J_H^c would be $-[0.1 \text{ (cal/cm}^2\text{-min)/}(^\circ/\text{cm})] (1^\circ/\text{cm})$, or $-0.1 \text{ cal/cm}^2\text{-min}$ by Equation 8.5. Such a heat flux into the soil can lead to the daytime heat storage calculated above, 40 cal/cm^2 , in $(40 \text{ cal/cm}^2)/(0.1 \text{ cal/cm}^2\text{-min})$, or 400 min, which is about 7 hr.

Soil energy balance. The components of the energy balance for the soil surface are similar to those for leaves (cf. Eqs. 7.22 and 7.23). However, we must also take into consideration the heat conducted into the soil itself (Eq. 8.5), which leads to a gradual temperature change of its upper layers.

The absorption and emission of radiation takes place in the upper mm or so of the soil. Using the symbols introduced in the previous chapter (Eqs. 7.24 through 7.27), the net radiation balance for the ground is $a(1 + r)(S^{\text{direct}} + S^{\text{cloud}} + S^{\text{sky}}) + a_{\text{IR}}\sigma(T^{\text{sur}})^4 - e_{\text{IR}}\sigma(T^{\text{soil}})^4$, where the values of all parameters are those at the soil surface. (If the soil is exposed directly to the sky, T^{sur} should be replaced by T^{sky} for the incident IR.) For a soil exposed to direct sunlight, the net energy input by radiation can be quite large—the temperature of the uppermost part of the crust can exceed 70° in a desert.

Let us now consider the flux for heat conduction between the overlying air and the soil as well as the heat flux accompanying water evaporation in the soil. The heat conducted across the relatively still air next to the soil surface is equal to $-K^{\text{air}} \partial T/\partial z$, where K^{air} is the thermal conductivity coefficient of air at the local temperature (cf. Eq. 7.28, which indicates that the heat conducted from both sides of a leaf is $-2K^{\text{air}} \partial T/\partial x$). The heat conducted *within* the soil can be calculated using Equation 8.5 ($J_H^c = -K^{\text{soil}} \partial T/\partial z$). For a given layer of soil where the water vapor flux changes by ΔJ_{wv} , the heat loss accompanying water evaporation (or heat gain accompanying condensation) equals $\Delta J_{wv} H_{\text{vap}}$, where H_{vap} is the heat necessary to evaporate a unit amount of water at the local soil temperature (cf. Eq. 7.29, $J_H^T = J_{wv} H_{\text{vap}}$). Except in the upper mm or so, the main energy flux in the soil is for heat conduction, not for radiation or for phase changes of water. The flux of water in the soil, discussed below, can also represent an important means of heat movement.

Soil water potential. The predominant influence on the soil water potential is usually the many air-liquid interfaces present in the soil (Fig. 8.4). Of course, the soil water contains dissolved solutes, which generally lead

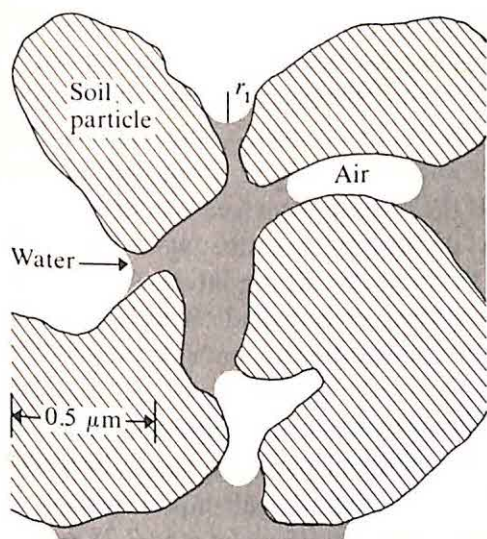


Figure 8.4

Schematic indication of the gaseous, liquid, and solid phases in a soil. The radii of curvature at the air-water interfaces help determine the negative hydrostatic pressures or tensions in the liquid phase.

to an osmotic pressure (π^{soil}) between 0.1 and 2 bars; the magnitude of π^{soil} depends on the relative wetness of the soil, which varies greatly. Because of the relatively low osmotic pressures, we will often refer to the solution in the soil as "water." In contrast to the generally small values for π^{soil} , the many interfaces present in the rather small soil pores can lead to hydrostatic pressures of -20 bars and even more negative ones. Such negative hydrostatic pressures are often referred to as (positive) matric potentials (cf. p. 69).

The surfaces of the air-water interfaces in the pores between the soil particles are usually concave when viewed from the air side, just as for capillaries (cf. Fig. 2.2). However, the surfaces generally are not spherical or otherwise regularly shaped (the same restriction also applies to the air-liquid interfaces in the pores in the cell wall). Nevertheless, it is possible to define two principal radii of curvature for a surface. Let us designate these radii, which occur in planes perpendicular to each other and to the liquid surface, by r_1 and r_2 . The hydrostatic pressure in the soil water is then:

$$P = -\sigma \left(\frac{1}{r_1} + \frac{1}{r_2} \right) \quad (8.6)$$

where σ is the surface tension at an air-liquid interface.* (The negative

* To connect Equation 8.6 with Equation 2.22 ($P = -2\sigma \cos \alpha / r$, where r is the radius of the capillary), note that for a cylindrical capillary the two principal radii of the surface are the same ($r_1 = r_2$), hence the factor $(1/r_1 + 1/r_2)$ is $2/r_1$, which equals $2 \cos \alpha / r$.

hydrostatic pressure or tension described by Eq. 8.6 and resulting from the presence of air-water interfaces is often called the *soil matric potential*.)

Instead of being concave, the water surface between certain soil particles may assume a nearly cylindrical shape, i.e., like a trough. This means that one of the radii of curvature becomes infinite, e.g., $r_2 = \infty$; in such a case, the tension is σ/r_1 by Equation 8.6. If the air-liquid surface is convex when viewed from the air side, the radii as defined are negative; we would then have a positive hydrostatic pressure in the water. In the intermediate case, viz., one radius positive and one negative (a so-called "saddle-shaped" surface), whether the pressure is positive or negative depends on the relative sizes of the two radii of curvature.

Let us now estimate the hydrostatic pressure in the soil water within a wedge-shaped crevice between two adjacent soil particles, as is illustrated at the top of Figure 8.4. We will assume that the air-liquid surface is cylindrical, so that r_2 equals ∞ , while r_1 is $0.1 \mu\text{m}$. If we let σ be 7.28×10^{-5} bar-cm, the value for pure water at 20° (App. II), then by Equation 8.6 the hydrostatic pressure is $-(7.28 \times 10^{-5} \text{ bar-cm})/(10^{-5} \text{ cm})$, or -7 bars. As the amount of soil water decreases, the air-water surface retreats into the crevice between the particles, the radius of curvature becomes less, and the pressure accordingly becomes more negative. Since by Equation 2.13 Ψ^{soil} equals $P^{\text{soil}} - \pi^{\text{soil}} + \rho_w gh$, the soil water potential also becomes more negative as water is lost from such crevices.

Darcy's law. Darcy in 1856 recognized that the flow of water through the soil was driven by a gradient in hydrostatic pressure. We can represent this relation, known as Darcy's law, by the following expression:

$$J_v = -L^{\text{soil}} \frac{\partial P^{\text{soil}}}{\partial x} \quad (8.7)$$

where J_v is the volume of solution crossing unit area in unit time and L^{soil} is the soil hydraulic conductivity coefficient.

Although Equation 8.7 is in a familiar form (flux equals a proportionality coefficient times a force), we have used $-\partial P^{\text{soil}}/\partial x$ instead of the possibly more general force, $-\partial \Psi^{\text{soil}}/\partial x$ ($\Psi^{\text{soil}} = P^{\text{soil}} - \pi^{\text{soil}} + \rho_w gh$, Eq. 2.13). In Chapter 3 we derived an expression for J_v that incorporated a reflection coefficient, $J_v = L_p (\Delta P - \sigma \Delta \pi)$ (Eq. 3.38). When σ is zero, as occurs for a porous barrier like soil, $\Delta \pi$ does not lead to any volume flux. Hence, we do not expect $\partial \pi^{\text{soil}}/\partial x$ to influence the movement of water in the soil, and it is not included in Darcy's law. Since $\partial(\rho_w gh)/\partial x$ is not incorporated into Equation 8.7, the indicated form of Darcy's law really applies only to horizontal flow in the soil ($\partial h/\partial x = 0$ when x is in a horizontal direction).

Actually, $\rho_w g$ is only 0.1 bar/m (App. II), so that changes of $\rho_w g h$ in the vicinity of a root are relatively small. However, for percolation of water down appreciable distances into the soil, P^{soil} in Equation 8.7 should be replaced by $P^{\text{soil}} + \rho_w g h$. In fact, for drainage of very wet soil the gravitational term can be the dominant factor in Darcy's law.

Soil hydraulic conductivity coefficient. L^{soil} in Equation 8.7 does not have the same units as L_p in Equation 3.38 [$J_v = L_p (\Delta P - \sigma \Delta \pi)$] or L_w in Equation 2.23 [$J_{v,w} = L_w (\Psi^o - \Psi^i)$]. L_p and L_w have units of volume flux/pressure, such as cm/sec-bar. However, L^{soil} in Equation 8.7 has units of volume flux/pressure gradient, such as (cm/sec)/(bar/cm), which is cm²/sec-bar. We could use other self-consistent sets of units for Darcy's law; indeed, the soil hydraulic conductivity coefficient has been expressed in many different ways.

The soil hydraulic conductivity coefficient depends on the geometry of the pores in the soil. For geometrically similar pore shapes, and ignoring certain surface effects, the hydraulic conductivity coefficient is approximately proportional to the square of the pore size. However, the pores are so complex in shape that, in general, we cannot directly calculate L^{soil} . As soil dries, its water potential decreases and P^{soil} becomes less. When P^{soil} decreases below the minimum hydrostatic pressure that can occur in some (fairly large) pores, water flows out of them, but it will remain in pores which have smaller dimensions and which can therefore have even more negative pressures—cf. Equation 8.6, $P = -\sigma/(r_1 + r_2)$. Not only is the higher conductivity of the larger pores thus lost, but the remaining pathway for water flow becomes more tortuous, and so L^{soil} decreases as the soil dries out.

The soil hydraulic conductivity coefficient can be 10⁻⁸ cm²/sec-bar or lower for a dry nonporous soil, and 10⁻⁴ cm²/sec-bar or higher for a wet porous one. For a porous clay whose pores are nearly filled with water, L^{soil} is usually 10⁻⁴ to 10⁻¹ cm²/sec-bar, while for a water-saturated sandy soil, the soil hydraulic conductivity coefficient is generally 1–10 cm²/sec-bar (see Childs; Hillel, and Rose). (We will reconsider L^{soil} in terms of Poiseuille flow on p. 392.)

The ground is often covered by a rather dry crust where the soil hydraulic conductivity coefficient is quite low. Specifically, L^{soil} may average 10⁻⁷ cm²/sec-bar in the upper 0.5 cm of the soil. If P^{soil} is -7 bars near the surface and -2 bars 0.5 cm beneath it, then by Darcy's law (Eq. 8.7) the flux of water would be $-(10^{-7} \text{ cm}^2/\text{sec-bar}) [-7 \text{ bars} - (-2 \text{ bars})]/(0.5 \text{ cm})$, or $1 \times 10^{-6} \text{ cm}^3/\text{cm}^2\text{-sec}$. This water flux directed vertically upward at the soil surface is equal to $(1 \times 10^{-6} \text{ cm}^3/\text{cm}^2\text{-sec})(1 \text{ mole}/18 \text{ cm}^3)$, or 0.06

$\mu\text{mole}/\text{cm}^2\text{-sec}$. In the previous section we indicated that J_{wv} emanating from a shaded soil is generally $0.02\text{--}0.1 \mu\text{mole}/\text{cm}^2\text{-sec}$, so that our calculated flux is consistent with the range of measured values.

During a rainstorm the upper part of the soil can become nearly saturated with water. As a result, L^{soil} there might increase a millionfold from $10^{-7} \text{ cm}^2/\text{sec-bar}$ all the way up to $0.1 \text{ cm}^2/\text{sec-bar}$. This then facilitates the entry or infiltration of water into the soil, often corresponding to a flux of $20 \mu\text{moles}$ of water/ $\text{cm}^2\text{-sec}$. Such a flux equals $(20 \times 10^{-6} \text{ mole}/\text{cm}^2\text{-sec})(18 \text{ cm}^3/\text{mole})(3600 \text{ sec/hr})$, or 1.3 cm of water/hr. The infiltration of a depth of water of 1.3 cm/hr can be maintained for a few hours by many soils (see Childs; Hillel; Slatyer, and Taylor and Ashcroft).

Besides moving as a liquid, water can also move as a vapor in the soil. Since water is continually evaporating from and condensing onto the many air-liquid interfaces in the soil, such movement can be relatively important, especially for dry soils where the liquid phases are very discontinuous. The saturation values for water vapor partial pressures or concentrations increase nearly exponentially with temperature.* Since the air between the soil particles is generally nearly saturated with water vapor, the amount of water vapor in the soil air increases rapidly with the soil temperature. Consequently, the movement of water in the form of vapor tends to be greater at higher soil temperatures.

Fluxes for cylindrical symmetry. So far in this text we have been considering one-dimensional cases in Cartesian coordinates, while the flux of soil water toward a small root involves cylindrical geometry. This seemingly minor geometrical change causes a considerable increase in the complexity of the equations. Here we will restrict our attention to the steady state condition where the fluxes do not change with time. Also, we will handle the cylindrically symmetric case where the fluxes and forces depend only on the radial distance r from the axis of the cylinder, and not on any angle or on the location along the cylinder.

For a cylindrical surface of length l along the axis, the total volume of solution crossing per unit time is $J_v 2\pi r l$, where J_v is the volume flux directed radially at a distance r from the axis of the cylinder. In the steady state $J_v 2\pi r l$ is constant and so the magnitude of the flux depends inversely on the radial distance. When L^{soil} is constant, we can represent the flux for

* It may be helpful for understanding this temperature dependence to note that the fraction of molecules in the upper part of the Boltzmann distribution (p. 121) which have a high enough energy to escape from the liquid increases exponentially with temperature.

the cylindrically symmetric case as

$$J_v = \frac{1}{r} \frac{L^{\text{soil}} (P_a - P_b)}{\ln (r_a/r_b)} \quad (8.8)$$

where P_a is the hydrostatic pressure at a distance r_a from the axis of the cylinder, and P_b is the value at r_b . J_v is considered to be positive when the net flux is directed into the root, as occurs when the hydrostatic pressure is higher ($P_a > P_b$) the further we are from the root ($r_a > r_b$). Equation 8.8 represents a quite general form for steady state cases with cylindrical symmetry, e.g., Fick's first law (p. 11) then is $J_j = (1/r) D_j (c_j^a - c_j^b) / \ln (r_a/r_b)$.

The uptake of water by a young root 1 mm in diameter is usually 0.1–0.5 ml/day per cm of root length (see Kramer, as well as Slatyer). An uptake of 0.3 ml/day corresponds to (0.3 cm³/day) (1 day/86,400 sec), or 3.5×10^{-6} cm³/sec. This occurs over a root surface area ($2\pi r l$) of (2π) (0.05 cm) (1 cm), or 0.31 cm² in the present case, and thus J_v at the root surface is $(3.5 \times 10^{-6} \text{ cm}^3/\text{sec}) / (0.31 \text{ cm}^2)$, or 1.1×10^{-5} cm/sec. The influx of water per unit root surface area is enhanced by the root hairs (p. 7). These protrusions from the epidermal cells are often about 12 μ m in diameter, up to 1 mm long, and vary from a few to over 1000/cm². J_v is decreased for older roots whose outer tissue often becomes extensively cutinized and suberized.

For representative root spacing in a soil, the water may move toward a root over a radial distance of about 1 cm. What drop in hydrostatic pressure might we expect over this interval? Let us assume that Ψ_a^{soil} at r_a equal to 1.05 cm is -3 bars, made up of an osmotic pressure of 1 bar and a P_a^{soil} of -2 bars ($\Psi = P - \pi + \rho_w g h$, Eq. 2.13). We will let L^{soil} be 10^{-6} cm²/sec-bar, as might apply to a loam of moderately low water content, and we will suppose that J_v at the surface of a root 1 mm in diameter (i.e., $r_b = 0.05$ cm) is 1.1×10^{-5} cm/sec. Using Equation 8.8, we calculate that P_b^{soil} near the root would equal $-(0.05 \text{ cm})(1.1 \times 10^{-5} \text{ cm/sec}) [\ln (1.05 \text{ cm}/0.05 \text{ cm})] / (10^{-6} \text{ cm}^2/\text{sec-bar}) + (-2 \text{ bars})$, or $-0.55 \ln (21) - 2$, which is -4 bars. Thus, the hydrostatic pressure drops 2 bars across a distance of 1 cm in the soil next to the root. Assuming that the solute content of the soil water does not change appreciably over this interval, π_b^{soil} is 1 bar and thus Ψ_b^{soil} adjacent to the root is $-4 - 1$, or -5 bars (cf. Table 8.2).

When the soil dries out, L^{soil} decreases; therefore, the drops in hydrostatic pressure and water potential would have to be larger in order to maintain a given volume flux toward a root. For example, L^{soil} is rather high for

a wet soil with a Ψ^{soil} of -0.2 bar; ΔP^{soil} over a radial distance of 1 cm from a root is generally 0.1 bar or less in such a case. To maintain the same J_v for Ψ^{soil} equal to -15 bars in the bulk soil, a drop in P^{soil} of about 10 bars might occur over the 1 cm interval adjacent to the root.

Water movement in the xylem and phloem

Under usual conditions, essentially all of the water entering a land plant comes from the soil by way of the root. The water is then conducted to the other parts of the plant mainly by the xylem. To reach the xylem in the mature part of a root, water must cross the root epidermis, the cortex, and then the endodermis. We will briefly describe the various cells encountered by water as it moves from the soil to the root xylem (see Fig. 1.4) and then discuss the dependence of water flow in the xylem on the gradient in hydrostatic pressure. Flow in the phloem may also depend on $\partial P/\partial x$, although osmotic pressures are also important for interpreting water movement in this other "circulatory" system.

Root tissues. Longitudinal and cross-sectional views near the tip of a root are presented in Figure 1.4, indicating the various types of cells which can act as barriers to flow. Water may fairly easily traverse the single-cell layer of the root epidermis to reach the cortex. The root cortex often consists of 5–10 cell layers, the cytoplasm of adjacent cells being continuous because of the plasmodesmata. The collective protoplasm of the interconnected cells is referred to as the *symplasm* (see Ch. 1). In the symplasm, permeability barriers in the form of plasmalemmas and cell walls do not have to be surmounted in order for diffusion to occur from cell to cell, and this facilitates the movement of water and solutes across the cortex. Much of the water flow across the root cortex actually occurs in an alternative pathway, the cell walls.

Along that part of a root where cells have differentiated but where there is little root thickening due to secondary conducting tissue, an endodermis occurs inside the cortex (Fig. 1.4 and p. 8). Upon reaching the endodermis, the cell wall pathway for water movement is blocked by the casparian strip. This is a continuous band around the radial walls of the endodermal cells; it blocks those walls in which water and solutes could have passed across the endodermal layer. The casparian strip is impregnated with suberin (a polymer of fatty acids) and lignin. It is generally considered part of the primary cell wall, but suberin and lignin are also deposited in the secondary wall (which often becomes quite thick) and all the way across the middle

lamella. Water and ions therefore cannot flow across the endodermal cells in their cell walls but rather must move through their cytoplasm. Thus, the endodermis, with its casparian strip, serves to regulate the passage of solutes and water from the cortex to the xylem in the affected parts of the root. Inside the endodermis are parenchyma cells known as the *pericycle*, which surrounds the vascular tissue containing the conducting vessels of the xylem and the phloem (cf. Fig. 1.4). During water movement through a plant accompanying transpiration, the hydrostatic pressure in the root xylem is reduced, and can become quite negative. This decreases the water potential ($\Psi = P - \pi + \rho_w gh$, Eq. 2.13) in the xylem and promotes water movement from the soil down a water potential gradient to the root xylem.

The xylem. Before discussing the characteristics of flow in the xylem, we will briefly review some of the anatomical features of its cells. In general, the conducting xylem elements have thick, lignified secondary walls and contain no protoplasts, i.e., the xylem cells serve their special function of providing the plant with a low resistance conduit only when they are dead.

Two types of conducting cells are distinguished in the xylem: the vessel members (found in angiosperms) and the phylogenetically more primitive tracheids (in angiosperms, gymnosperms, and the lower vascular plants). Tracheids are typically tapered at the ends, while the generally shorter and broader vessel members often abut each other with rather blunt ends. The end cell walls of the vessel members are perforated, so that the vessel members arranged end-to-end form a continuous tube called a *xylem vessel*. The end cell wall of a vessel member bearing the holes is referred to as the *perforation plate*; a simple perforation plate (Fig. 1.3) essentially eliminates the end walls between the individual members in a vessel. Although xylem elements actually vary considerably in their widths (from $10\ \mu\text{m}$ all the way up to $500\ \mu\text{m}$), we will represent them by cylinders with radii of $20\ \mu\text{m}$ for purposes of calculation. Conducting cells of the xylem generally range in length from a few hundred microns to a few mm.

Besides vessel members and tracheids, other types of cells in the xylem are fiber and parenchyma cells (Fig. 1.3). Xylem fibers, which contribute to the structural support of a plant, are long thin cells having heavily lignified cell walls; they are devoid of protoplasts at maturity. The living parenchyma cells in the xylem are important for the storage of carbohydrates and for the lateral movement of water and solutes into and out of the conducting cells.

Poiseuille's law. In order to describe fluid movement in the xylem quantitatively, we need to relate the flow to the particular driving force causing the motion. For cylindrical tubes, an appropriate relationship is the one

determined experimentally by Hagen in 1839, and independently by Poiseuille in 1840. They found that the volume of fluid moving in unit time along a cylinder is proportional to the fourth power of its radius, and that the movement depends linearly on the drop in hydrostatic pressure. Wiedemann in 1856 showed that this rate of volume movement equals $-(\pi r^4/8\eta) \partial P/\partial x$, where r is the radius of the cylinder, η is the viscosity of the solution, and $-\partial P/\partial x$ is the negative gradient of the hydrostatic pressure. Throughout this text we have been concerned with the volume flowing per unit time and area, J_v . For flow in a cylinder of radius r , and hence area πr^2 , J_v would be

$$J_v = -\frac{r^2}{8\eta} \frac{\partial P}{\partial x} \quad (8.9)$$

Since positive flow ($J_v > 0$) occurs in the direction of decreasing hydrostatic pressure ($\partial P/\partial x < 0$), the minus sign is necessary in Equation 8.9.

Equation 8.9 is generally referred to as *Poiseuille's law*, and certain of its features deserve special emphasis. It was derived assuming that the fluid in the cylinder moves in layers, or *laminas*, each layer gliding over the adjacent one (see Streeter). Such laminar movement occurs only if flow is slow enough to meet a criterion deduced by Reynolds in 1883. That is, the dimensionless quantity $\rho J_v r / \eta$ (known as the Reynolds number) must be less than 2000 (ρ is the solution density). Otherwise, a transition to turbulent flow occurs, and Equation 8.9 no longer is valid. Another important point is that the fluid in Poiseuille (laminar) flow is actually stationary at the wall of the cylinder; the velocity of solution flow increases in a parabolic fashion to a maximum value in the center of the tube. Thus, the flux in Equation 8.9 is actually the mean flow averaged over the entire cross-section of cylinders of radius r . The cgs unit for viscosity η is the *poise*, which has dimensions of dyne-sec/cm²; the viscosity of water at 20°, for instance, is 0.01002 poise. (Values of η_w at other temperatures are given in App. II.)

As we have already indicated, the volume fluxes described by both Poiseuille's law [$J_v = -(r^2/8\eta) \partial P/\partial x$, Eq. 8.9] and Darcy's law ($J_v = -L^{\text{soil}} \partial P^{\text{soil}}/\partial x$, Eq. 8.7) depend on the negative gradient of the hydrostatic pressure. Can we establish any correlation between these two equations? If the soil pores were cylinders of radius r , all aligned in the same direction, we could have Poiseuille flow in the soil, in which case L^{soil} would equal $r^2/8\eta$ —we are assuming for the moment that the pores occupy the entire soil volume. When r is 1 μm and η equals 0.010 poise, $r^2/8\eta$ is $(1 \times 10^{-4} \text{ cm})^2 / [(8)(0.010 \text{ dyne-sec/cm}^2)]$, or $1.3 \times 10^{-7} \text{ cm}^4/\text{dyne-sec}$, which is equal to $0.13 \text{ cm}^2/\text{sec-bar}$ (1 bar = 10^6 dynes/cm², App. III). However, L^{soil} for a

water-filled porous clay with average pore radii of $1\text{ }\mu\text{m}$ is about $10^{-2}\text{ cm}^2/\text{sec-bar}$, which is about tenfold smaller than $r^2/8\eta$ calculated using the average pore radius. As a result, J_v in the soil is considerably less than for Poiseuille flow through pores of the same average radius. This is mainly because the soil pores are not in the shape of cylinders aligned in the direction of flow; also, the pores between soil particles occupy only about half of the soil volume.

Applications of Poiseuille's law. Next, we will use Poiseuille's law (Eq. 8.9) to estimate the pressure gradient necessary to cause a specified volume flow in the conducting cells of the xylem. The velocity of sap ascent in the xylem of a transpiring tree can be near 0.1 cm/sec , which equals 3.6 m/hr (see Briggs; Slatyer, and Zimmermann and Brown). On p. 86 we indicated that $\bar{v}_w$ equals the volume flow of water, J_{vw} ; the average velocity of the solution equals J_v , the volume flow of the solution.* Thus, J_v in the xylem of a transpiring tree might be 0.1 cm/sec . For a viscosity of 0.010 poise and a xylem element with a $20\text{ }\mu\text{m}$ radius, the pressure gradient required to satisfy Equation 8.9 is $-(8)(0.01\text{ dyne-sec/cm}^2)(0.1\text{ cm/sec})/(20 \times 10^{-4}\text{ cm})^2$ or $-2 \times 10^3\text{ dynes/cm}^3$. As expected, the pressure decreases along the direction of flow.

Since a bar is 10^6 dynes/cm^2 (App. III), the above $\partial P/\partial x$ satisfying Poiseuille's law equals $-2 \times 10^{-3}\text{ bar/cm}$, or -0.2 bar/m . This estimate is consistent with experimental observations of the $\partial P/\partial x$ accompanying solution flow in *horizontal* xylem vessels. For the vertical vessels in a tree, however, a static hydrostatic pressure gradient of -0.1 bar/m due to gravity exists even in the absence of flow.† For transpiring plants, the total $\partial P/\partial x$ in the vertical sections of the xylem is often about -0.3 bar/m . Based on the above calculations, this pressure gradient is sufficient to overcome gravity (-0.1 bar/m) and to cause Poiseuille flow in the xylem vessels (-0.2 bar/m is needed in the above example, where $r = 20\text{ }\mu\text{m}$). (See Briggs.)

We have been approximating xylem vessels by cylinders $20\text{ }\mu\text{m}$ in radius, as might be appropriate for diffuse-porous (small-porous) trees. For ring-porous (large-porous) trees, the mean radii of xylem vessels are often about

* In any case, for a dilute aqueous solution such as occurs in the xylem, the volume flow of solution (J_v) is essentially the same as the volume flow of water, J_{vw} (see also p. 143).

† We can appreciate this by considering a vertical column of pure water ($\pi = 0$) at equilibrium, where the water potential (Ψ) is $P + \rho_w gh$; $\rho_w gh$ increases vertically upward, and P must decrease by the same amount in order for Ψ to remain unchanged, as it does at equilibrium. Since $\rho_w g$ equals 0.098 bar/m (App. III), the additional pressure gradient caused by gravity amounts to -0.1 bar/m .

100 μm (see Zimmermann and Brown). For a given pressure gradient, Poiseuille's law (Eq. 8.9) indicates that a much larger velocity of xylem sap movement would occur in the ring-porous trees, as is indeed observed. Using our above values and a density of 1 g/cm^3 ($= 1 \text{ dyne-sec}^2/\text{cm}^4$), the Reynolds number ($\rho J_v r / \eta$) for the diffuse-porous case would be $(1 \text{ dyne-sec}^2/\text{cm}^4)(0.1 \text{ cm/sec})(2 \times 10^{-3} \text{ cm}) / (0.01 \text{ dyne-sec/cm}^2)$, or only 0.02. Such a low value indicates that no turbulence is expected. Even if r were fivefold larger and J_v were tenfold higher, as can occur in a ring-porous tree, the Reynolds number would still be far less than the value of 2000, where turbulence generally sets in.

It is interesting to estimate the sort of pressure gradient necessary to promote a given flow along a cell wall. This use of Poiseuille's law will allow us to compare such a pathway with water movement in the lumens of xylem vessels. Since the interfibrillar spaces, or interstices in a cell wall have diameters near 10 nm (100 Å), we will let r be 5 nm for purposes of calculation. (Complications due to the tortuosity of the aqueous channels through the interstices will be omitted here. Also, we will pretend that the pores occupy the entire cell wall.) For J_v equal to 0.1 cm/sec, Equation 8.9 indicates that the pressure gradient required for solution flow through the cell walls is $-(8)(0.01)(0.1)/(5 \times 10^{-7})^2$, or $-3.2 \times 10^{10} \text{ dynes/cm}^3$, which equals $-3 \times 10^6 \text{ bars/m}$. A $\partial P / \partial x$ of only -0.2 bar/m is needed for the same J_v in the xylem element having a 20 μm radius. Thus, the $\partial P / \partial x$ for Poiseuille flow through the small interstices of a cell wall is over 10^7 times greater than for the same flux through the lumen of the above xylem element. Because of the tremendous pressure gradients required to force water through the small interstices available for solution conduction in the cell wall, fluid could not flow rapidly enough up a tree in the cell walls—as has been suggested—to account for observed rates of water movement.

To help compare the relative effects on flow of a cell wall, a plasmalemma, and the lumen of a xylem vessel, we will calculate the hydrostatic pressure drops across each of them in a hypothetical case. Let us consider a xylem vessel 1000 μm (10^{-3} m) long and 20 μm in radius. We just calculated the pressure gradient necessary for a Poiseuille flow of 0.1 cm/sec in the lumen of this xylem vessel to be -0.2 bar/m , which amounts to $(-0.2 \text{ bar/m})(10^{-3} \text{ m})$, or $-2 \times 10^{-4} \text{ bar}$ for the 1000 μm length of the cell. Now let us suppose that a cell wall 5 μm thick is placed on either end of the cell. In the previous paragraph we calculated that it would take a pressure gradient of $-3 \times 10^6 \text{ bars/m}$ to cause a J_v of 0.1 cm/sec through the interstices of a cell wall. To cross this cell wall (a distance of 10 μm or 10^{-5} m , including both ends) would therefore require a pressure change of $(-3 \times 10^6$

bars/m)(10^{-5} m), or -30 bars. Finally, let us imagine that a plasmalemma with a hydraulic conductivity coefficient of 10^{-5} cm/sec-bar is also installed around the cell. Using Equation 3.38, $J_v = L_p(\Delta P - \sigma\Delta\pi)$, with J_v equal to 0.1 cm/sec, the difference in hydrostatic pressure required for such flow would be $(0.1 \text{ cm/sec})/(10^{-5} \text{ cm/sec-bar})$, or 10^4 bars, which means a total drop of 2×10^4 bars to traverse the plasmalemmas at each end of the cell. In summary, the pressure drops needed in this hypothetical case are 2×10^{-4} bar to flow through the lumen, 30 bars to cross the cell walls, and 2×10^4 bars to cross the membranes.

In fact, membranes generally serve as the main barrier to water flow across plant cells. The interstices of the cell walls provide a much easier pathway for solution flow (such as across the root cortex), while a hollow xylem vessel presents the least obstacle to flow (such as up the stem). Consequently, the evolution of xylem provides a plant with a tube, or conduit, well-suited for moving water over long distances. The region of a plant made up of cell walls and the hollow xylem vessels is often given a special name, the *apoplast*. Water and the solutes it contains can move fairly readily in the apoplast, whereas they must cross a membrane in order to enter the symplast (symplasm), the interconnected cytoplasm of the cells.

The phloem. Our discussion of water movement in plants would not be complete without a consideration of transport in the phloem. Water and solute movement in this vascular tissue involves cooperative interactions among several types of cells. Furthermore, the conducting cells of the phloem generally have a high internal hydrostatic pressure, which often leads to artifacts during cytological investigations. In part because of the observational difficulties involved, there is a lack of agreement as to the actual internal structure of the cells and the mechanism for solute movement in the phloem. In our brief treatment here we will be primarily concerned with certain anatomical features of the phloem cells and an overall description of flow. Although a final decision on mechanism must await future research, certain implications of a pressure-driven flow will be presented.

The conducting cells of the phloem are the sieve cells in lower vascular plants and gymnosperms, and the more advanced sieve-tube members in angiosperms (Fig. 1.3). Both types of cells are collectively called *sieve elements*. Mature sieve elements almost invariably have lost their nuclei, and apparently the tonoplast has broken down so that there is no large central vacuole. However, unlike the conducting elements of the xylem, the sieve elements contain cytoplasm and are living. The sieve elements in most plants vary from 0.1 to several mm in length, and tend to be somewhat longer in gymnosperms than in angiosperms. Typical radii are $10\text{--}30 \mu\text{m}$.

Specialized cells are generally found adjacent to the sieve elements, called *companion cells* in angiosperms (see Fig. 1.3) and *albuminous cells* in gymnosperms. Although their actual functions are still unclear, such cells are metabolically related to the conducting cells and may supply them with carbohydrates and other materials. For example, companion and albuminous cells accumulate sugars and other solutes which may either passively diffuse, e.g., through the plasmodesmata which are present, or be actively transported into the sieve elements (see Crafts and Crisp, and Esau).

Sieve-tube members usually abut end-to-end to form the *sieve tubes*. The pair of generally inclined end walls between two sequential sieve-tube members form the so-called *sieve plate* (Fig. 1.3). Sieve plates have many pores, ranging in diameter from less than $1\text{ }\mu\text{m}$ up to about $5\text{ }\mu\text{m}$, and ordinarily have strands of cytoplasmic material passing through them. The pores are lined, not crossed, by the plasmalemmas. Solution moving in the phloem therefore does not have to cross any membranes as it flows from cell to cell along a sieve tube. A distinguishing feature of sieve-tube members is the presence of "P protein" (formerly termed *slime*), which often occurs in a fibrillar form. Although the exact functions of this material are unclear, it plugs the sieve plate pores in the event that the sieve tube is injured. For instance, when the phloem is opened by cutting, its positive hydrostatic pressure forces the contents of the conducting cells toward the incision, carrying the P-protein into the sieve plate pores. As a further wound response, callose (a glucose polysaccharide) can also be deposited in the sieve plate pores, thereby closing them within a matter of minutes following injury.

Phloem contents and velocity of movement. A rather elegant way of studying the contents of certain sieve elements is by means of aphid stylets. An aphid feeds on the phloem by inserting its stylet into an individual sieve element. After the aphid has been anesthetized and its body removed, the remaining mouth part forms a tube that leads the phloem solution from the sieve element to the outside. Using this technique to extract phloem solution, over 90% of the solutes in the sieve element are found to be carbohydrates, mainly sucrose and some other oligosaccharides. The concentration of sucrose is generally 0.2–0.7 M; values near 0.3 M are typical for small plants, while some tall trees have sucrose concentrations near the upper limit. The types and concentrations of the solutes exhibit diurnal and seasonal variations, and also depend on the tissues that the phloem solution is flowing toward or away from. For example, the solution in the phloem moving out of senescent leaves is low in sucrose but contains an appreciable concentration of amino acids and amides, sometimes as high as 0.5 M—an amino acid concentration near 0.05 M is representative of

phloem solution in general. The initial movement of ions from the root to the rest of the plant occurs mainly in the xylem; subsequent recirculation can take place in the phloem, such as the movement of ions out of leaves just before their abscission. (See Crafts and Crisp.)

Solutes can move rapidly in the phloem in either direction, flow being toward regions of lower osmotic pressure. Thus, photosynthetic products move from the leaves to storage tissues in the stem and root where they are generally converted to starch. At other times, sugars produced from the hydrolysis of such starch may move in the opposite direction, from the storage tissue to meristematic areas at the top of the plant. The velocity of solute movement is ordinarily 0.2–1 m/hr, the rate varying, among other things, with the plant species and vigor of growth (see Canny; and Crafts and Crisp). Although a particular sugar generally moves in the phloem in the direction of a decrease in its concentration, diffusion is not the mechanism. First, the rate of solute movement far exceeds that of diffusion (see Ch. 1). Second, when a radioactive solute is introduced into a plane in a sieve element, the “front” moves with a rather constant velocity (distance moved is proportional to time), whereas in a one-dimensional diffusional process, distance moved is proportional to the square root of time in such a case ($x_e^2 = 4D_jt$, Eq. 1.6).

Instead of the solute velocity in the phloem, we are often more interested in how much dry matter is actually translocated. For example, if the sieve elements contain 0.3 M sucrose moving at an average velocity of 0.6 m/hr, what would be the transfer rate of sucrose in g/cm²-hr? By Equation 3.6 ($J_j = \bar{v}_j c_j$), the flux of sucrose is (60 cm/hr)(0.3 mole/1000 cm³), or 0.018 mole/cm²-hr. Since sucrose has a molecular weight of 342 g/mole, this J_j corresponds to (0.018 mole/cm²-hr)(342 g/mole), or 6 g/cm²-hr. In the present example, the flow is per cm² of sieve-tube *lumens*; the rate of flow per unit area of *phloem tissue* would be less by the ratio of the lumen cross-sectional area to the total phloem cross-sectional area.

Mechanism of phloem flow. What causes the movement of solutes in the phloem? Primarily because of observational difficulties, this question has proved hard to answer. A secondary complication is that water may readily enter and leave the various types of cells in the phloem and surrounding tissue. The phloem therefore cannot be viewed as an isolated independent system. For example, when the water potential in the xylem decreases, as occurs during rapid transpiration, the velocity of fluid movement in the phloem generally becomes less. Some water may move upward in the xylem and, later, downward in the phloem; but this is not the whole story, since movement in the phloem can be in either direction.

Around 1930, E. Münch proposed that fluid movement in the phloem was caused by a gradient in hydrostatic pressure. This would lead to flow analogous to that in the xylem as described by Poiseuille's law (Eq. 8.9), $J_v = -(r^2/8\eta)(\partial P/\partial x)$. To examine this hypothesis, we will assume that the average velocity of flow in the lumen of the conducting cells of the phloem (J_v) is 0.6 m/hr, or 0.017 cm/sec. Our sieve-tube members will be 15 μm in radius and 0.1 cm long, and the sieve plates 5 μm thick, with 1.5 μm radius pores covering one-third of their surface area. Based on the relative areas available for conduction, J_v is three times as high in the sieve plates as in the lumen of the sieve tube, viz., 0.051 cm/sec across the sieve plates. The viscosity of the solution in a sieve tube is greater than that of water, e.g., η^{phloem} may be near 0.015 poise at 20°, the value for 0.4 M sucrose ($\eta_w = 0.010$ poise at 20°, where poise = dyne-sec/cm², Apps. II and III). Assuming that η^{phloem} is 0.015 poise, the pressure change per cell expected from Poiseuille's law would be $-[(8)(0.015 \text{ dyne-sec/cm}^2)(0.017 \text{ cm/sec})/(15 \times 10^{-4} \text{ cm})^2](0.1 \text{ cm})$ in the lumen and $-[(8)(0.015 \text{ dyne-sec/cm}^2)(0.051 \text{ cm/sec})/(1.5 \times 10^{-4} \text{ cm})^2](5 \times 10^{-4} \text{ cm})$ across the sieve plate, which is $-90 - 140$ or a total of -230 dynes/cm^2 , which equals $-2.3 \times 10^{-4} \text{ bar}$. For cells 0.1 cm long, the pressure gradient needed to cause Poiseuille flow is then $(1000 \text{ cells/m})(-2.3 \times 10^{-4} \text{ bar/cell})$, or -0.2 bar/m .

In the above case, slightly over half of the hydrostatic pressure gradient in the phloem is necessary to overcome the resistance of the sieve plate pores. When the end walls are steeply inclined to the axis of the sieve element, the pores of the sieve plate can actually occupy an area greater than that of the cross-section of the sieve tube. This causes J_v in the pores to be less than in the lumen, which tends to reduce the resistance to flow in the phloem.

Possible values for components of the water potential in the phloem. We will now examine some of the consequences of the plausible, but unproved hypothesis of a pressure-driven flow in the phloem. We will let the water potential in the xylem be -6 bars at ground level and -8 bars 10 m above the ground (see Table 8.2 and Fig. 8.5). Since there is no evidence for active transport of water across membranes into the phloem, the water potential in the phloem 10 m above the ground ($\Psi^{\text{phloem}_{10 \text{ m}}}$) must be lower than -8 bars if water is to enter passively—water entering the phloem in the leaves can come from the xylem. The water potential there might be -10 bars, for example. By analogous reasoning, Ψ^{phloem} in the root must be higher than Ψ^{xylem} if water is to spontaneously leave the phloem in the region where the solutes, such as sucrose, are removed; an appropriate value for the water potential in the phloem at ground level ($\Psi^{\text{phloem}_0 \text{ m}}$) might be -4 bars. Water may then flow down the phloem from a water potential of -10 bars

to one of -4 bars. This may seem at first like an energetically uphill movement. However, as in the xylem, the term representing the force on solution moving within the phloem is ΔP , not $\Delta \Psi$.

The fact that the drop in hydrostatic pressure, not the change in water potential, represents the driving force along the phloem deserves special emphasis. By Equation 3.38, J_V equals $L_P (\Delta P - \sigma \Delta \pi)$, where σ within the phloem is zero because there are no membranes intervening between sequential members of a sieve tube. Differences in the osmotic pressure at various locations in the phloem, which help determine the overall change in water potential, do not therefore directly affect the movement of solution *along* the phloem. On the other hand, substances entering or leaving the phloem generally pass across a plasmalemma, which most likely has a mean reflection coefficient close to unity for solutes such as sucrose. J_V given by Equation 3.38 is then $L_P (\Delta P - \Delta \pi)$, which equals $L_P \Delta \Psi$ by Equation 2.13 ($\Psi = P - \pi + \rho_w g h$). Consequently, the change in water potential probably does represent the driving force on water moving into or out of the phloem (cf. Fig. 8.5).

Next, we will estimate the various components of the water potential at the upper end of the phloem tissue under consideration (Fig. 8.5). The osmotic pressure in the phloem of a leaf 10 m above the ground ($\pi_{\text{phloem } 10 \text{ m}}$) might be due to the following solutes: 0.5 M sucrose, 0.1 M other sugars, 0.05 M amino acids, and 0.05 M inorganic ions. Using the Van't Hoff relation— $\pi_s = RT \sum_j c_j$ (Eq. 2.10), where RT is 24.37 liter-bars/mole at 20°

(App. III)—we estimate that the osmotic pressure in the phloem 10 m above the ground is $(24.37)(0.5 + 0.1 + 0.05 + 0.05)$, or 17 bars. Such a large osmotic pressure, caused by the high concentrations of sucrose and other solutes, suggests that active transport is necessary at some stage in order to move certain photosynthetic products from leaf mesophyll cells to the sieve elements of the phloem. From the definition of water potential, $\Psi = P - \pi + \rho_w g h$ (Eq. 2.13), we conclude that $P_{\text{phloem } 10 \text{ m}}$ must be $\Psi_{\text{phloem } 10 \text{ m}} + \pi_{\text{phloem } 10 \text{ m}} - \rho_w g h_{10 \text{ m}}$, which is $(-10) + (17) - (1)$, or 6 bars ($\rho_w g h_{10 \text{ m}} = 1$ bar, Table 8.2). This appreciable hydrostatic pressure would cause sieve-tube members to exude when cut, as in fact occurs.

As we indicated on p. 398, the hydrostatic pressure gradient might be -0.2 bar/m for Poiseuille flow in representative (horizontal) conducting cells of the phloem. P would therefore drop 2 bars for a distance of 10 m. Gravity also leads to a pressure gradient in a column of water (1 bar increase in P when descending 10 m, cf. p. 393), so that in the present case the ΔP needed to cause Poiseuille flow vertically *downward* is -1 bar. Thus, the

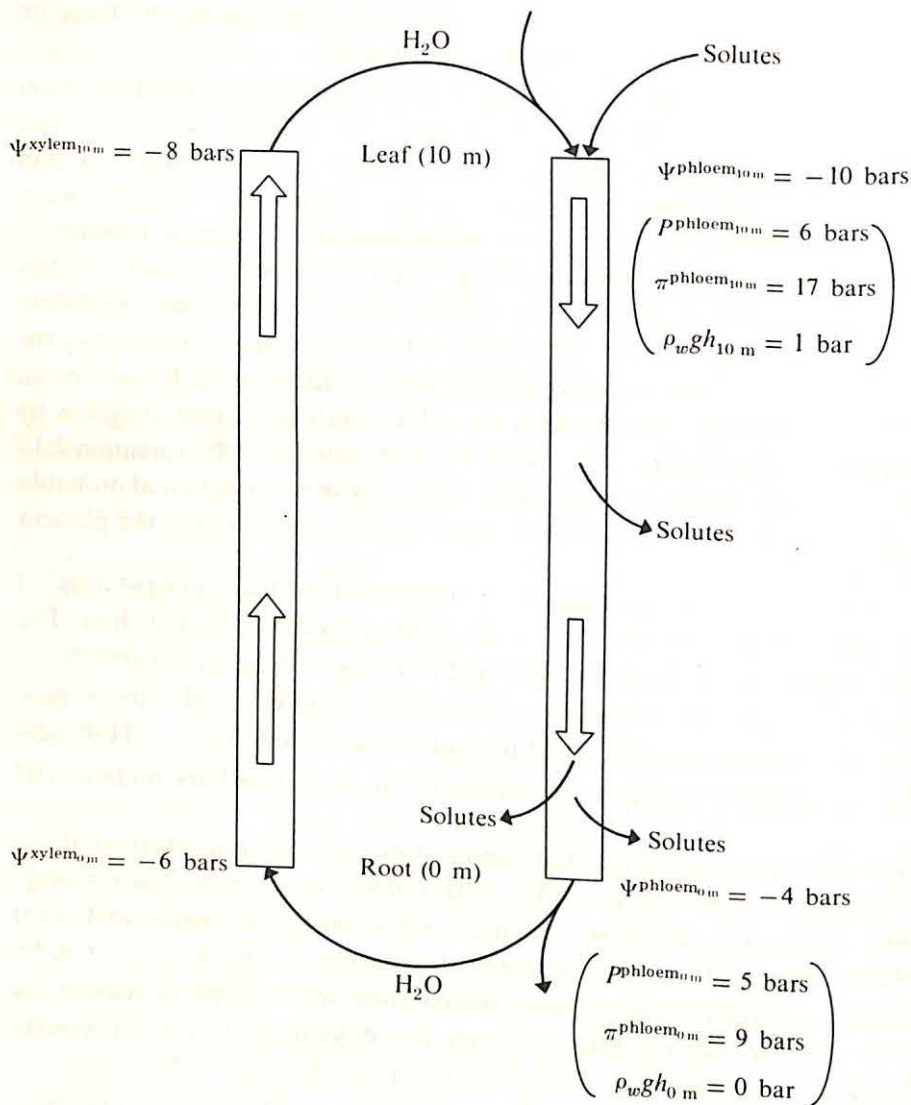


Figure 8.5

Hypothetical representation of phloem flow driven by a gradient in hydrostatic pressure. Water enters and leaves the phloem passively by moving toward regions of lower water potential. Solute either diffuses or is actively transported into and out of the sieve elements, leading to a decrease in the phloem osmotic pressure from 17 bars in the leaf to 9 bars in the root.

hydrostatic pressure in the phloem at ground level is 5 bars. The osmotic pressure there equals $P^{\text{phloem}}_m + \rho_w g h_{0m} - \Psi^{\text{phloem}}_m$ (Eq. 2.13), which is $(5) + (0) - (-4)$, or 9 bars ($\rho_w g h_{0m} = 0$ bar). The values of Ψ^{phloem} and its components when h equals 0 m and 10 m are summarized in Figure 8.5.

In our present example, the osmotic pressure of the phloem solution decreases from 17 bars in the leaf to 9 bars in the root (Fig. 8.5). Such a decrease is expected, based on the phloem's function of delivering photosynthetic products to different parts of the plant. Moreover, our calculations indicate that flow is in the direction of decreasing concentration, but that diffusion is not the mechanism. (Although the total concentration decreases in the direction of flow, this does not mean that each c_j necessarily does so.) Finally, we note the importance of removing solutes, either by active transport or by diffusion into the cells near the conducting cells of the phloem. The known involvement of metabolism in translocation in the phloem could be due to active transport of solutes into the phloem of the leaf (or other "source")—which is often referred to as "loading"—and/or their removal in the root (or other "sink"). Low concentrations of translocated solutes in the cells outside the phloem, which would favor the diffusion of these solutes out of the sieve elements, could also be maintained by metabolic conversions of phloem solutes, such as by starch formation in the cells adjacent to the conducting cells of the phloem. In any case, active loading or unloading of the phloem sets up a gradient in osmotic pressure, which in turn generates the hydrostatic pressure gradient that could lead to Poiseuille flow along the phloem. However, much remains to be learned about the actual details of fluid movement in the phloem.

The soil-plant-atmosphere continuum

As our final topic, we will examine certain aspects of the movement of water from the soil, through a plant, out into the surrounding atmosphere. During a growing season, about 100 times more water is transpired by a plant than remains in it. Thus, the amount of transpiration is actually a fairly accurate estimate of the water uptake by the roots. Although the rate that water crosses each section of the pathway is essentially the same in the steady state, the resistances and the areas across which water flows differ markedly for the various components.

Values of water potential components. Possible values for the water potential and its components in various parts of the soil-plant-atmosphere system are given in Table 8.2. The values do not apply to all plants, nor

Table 8.2

Representative values for the various components of the water potential in the soil-plant-atmosphere system. Ψ equals $P - \pi + \rho_w gh$ in the liquid phases (Eq. 2.13), and $(RT/\bar{V}_w) \ln (\% \text{ relative humidity}/100) + \rho_w gh$ in the gas phases (Eq. 2.21), all at 25°.

Location	P (bars)	$-\pi$ (bars)	$\rho_w gh$ (bars)	$\frac{RT}{\bar{V}_w} \ln \left(\frac{\% \text{ relative humidity}}{100} \right)$ (bars)	Ψ (bars)
Soil 0.5 cm below ground and 1 cm from root	-2	-1	0		-3
Soil adjacent to root	-4	-1	0		-5
Xylem of root near ground surface	-5	-1	0		-6
Xylem in leaf at 10 m above ground	-8	-1	1		-8
Vacuole of leaf mesophyll cell at 10 m	2	-11	1		-8
Cell wall of leaf mesophyll cell at 10 m	-4	-5	1		-8
Air in cell wall pores at 10 m (water vapor assumed to be in equilibrium with water in cell wall)			1	-9	-8
Air just inside stomata at 95% relative humidity			1	-70	-69
Air just outside stomata at 60% relative humidity			1	-702	-701
Air just across unstirred layer at 50% relative humidity			1	-951	-950

even to the same plant at all times. Rather, they serve to indicate representative contributions of P , π , $\rho_w gh$, and relative humidity to Ψ in various parts of the soil-plant-atmosphere continuum.

First, we will consider the soil water potential, Ψ^{soil} . As we indicated earlier in this chapter, Ψ^{soil} is usually dominated by P^{soil} , which is negative because of the surface tension effects at the numerous air-liquid interfaces present in the soil. The actual magnitude of the water potential varies greatly with environmental conditions and the type of soil. In freshly irrigated soil it may be about -0.1 bar, while permanent wilting of plants often occurs when the soil water potential decreases below about -15 bars.

The value of the soil water potential at which wilting of a plant occurs depends on the osmotic pressure in the vacuoles of its leaf cells, as the following argument will show. Let us consider the case in Table 8.2 where

the water potential in the vacuole of a leaf cell 10 m above the ground is initially -8 bars. As the soil dries out, Ψ^{soil} decreases and eventually becomes -10 bars. When the soil water potential becomes -10 bars, Ψ^{leaf} must be less than this in order for water movement to continue from the soil to the leaf. Ψ^{leaf} could be -10 bars when P^{vacuole} is 0 bar, $\rho_w gh^{\text{vacuole}}$ remains 1 bar, and π^{vacuole} remains 11 bars (the value in Table 8.2). (Actually, as the hydrostatic pressure in a leaf cell decreases, the elastic properties of the cell wall ensure that the cell will shrink somewhat, so that π^{vacuole} increases slightly.) Zero hydrostatic pressure in the vacuole means that the cell has lost its turgor, and the leaf wilts in response to this low Ψ^{soil} . For xerophytes in arid areas, the osmotic pressure in the leaves is often 25-50 bars under normal physiological conditions. The value of the soil water potential at which wilting occurs for such plants would be considerably lower (i.e., more negative) than for the plant indicated in Table 8.2. A high osmotic pressure in the vacuoles of the leaf cells can therefore be viewed as an adaptation to the low soil water potentials in an arid climate (see Slatyer).

If the soil dries out even further from the level causing wilting, and if water movement from the soil to the leaf still occurs, i.e., if Ψ^{leaf} is less than Ψ^{soil} , cellular water will be lost from the leaf. The vacuolar contents then become more concentrated, and π^{vacuole} increases. For instance, if Ψ^{leaf} became -20 bars for a Ψ^{soil} of -15 bars, the osmotic pressure in the vacuole of a leaf cell 10 m above the ground would be 21 bars, which represents a loss of nearly half of the cellular water (π^{vacuole} originally was 11 bars for this leaf cell, Table 8.2).

As we have already indicated, the driving force for water movement in the xylem is the negative gradient in hydrostatic pressure, which can lead to a flow describable by Poiseuille's law (Eq. 8.9). In Table 8.2, P^{xylem} decreases by 3 bars from the root to a leaf 10 m above the ground. The xylary sap, which contains chiefly water plus some minerals absorbed from the soil, usually does not have an osmotic pressure in excess of 2 bars. The hydrostatic pressure, on the other hand, can have much larger absolute values, and generally changes markedly during the day. When there is extremely rapid transpiration, large tensions (negative hydrostatic pressures) can develop in the xylem. These tensions are maintained by the cohesion of water molecules resulting from the intermolecular hydrogen bonding (Ch. 2). When transpiration ceases, as can occur at night or under conditions of very high relative humidity in the air surrounding the plant, the hydrostatic pressure in the xylem often becomes positive. This can cause guttation as xylem fluid is then exuded from the leaves.

The marked changes in hydrostatic pressure in the xylem can cause plants

to have observable diurnal fluctuations in their diameters. When the transpiration rate is high, the large tension within the xylem vessels is transmitted to the water in the cell walls of the xylem vessels, then to communicating water in adjacent cells, and eventually all the way across the stem. The decrease in hydrostatic pressure in its trunk can therefore cause a whole tree to contract during the day. At night, the hydrostatic pressure in the xylem may become positive, and the tree diameter then increases, generally by about 1%. Such changes in tree diameter, and therefore volume, represent net release of water during the day and storage at night. In fact, the diurnal change in water content of a plant can be equal to the amount of water transpired in 10 min during the day. These changes in water content correspond to a capacitance effect superimposed on the resistance network for water flow (see Jordan and Ritchie; Kozlowski, Vol. III; and Slatyer).

Water is conducted to and across the leaves in the xylem vessels. It then moves to the individual leaf cells, presumably by flowing mainly in the cell walls. The water potential is usually about the same in the vacuole, the cytoplasm, and the cell wall of a particular leaf mesophyll cell (cf. values in Table 8.2). If this were not the case, water would redistribute itself by flowing energetically downhill toward lower water potentials. Since the water in the cell wall pores can be in contact with air, where evaporation takes place, there is a volume flux along the interstices to replace lost water. Such flow can be approximately described by Poiseuille's law (Eq. 8.9), which indicates that a (small) hydrostatic pressure gradient exists across such cell walls.

As water continually evaporates from the cell walls of mesophyll cells, the accompanying solutes originally in the xylary sap are left behind and can accumulate in the cell wall water. Some solutes are of course needed for cell growth. For halophytes and xerophytes growing in soils of high salinity, excess inorganic ions are actively excreted from a leaf by salt glands on its surface (see Lüttge, and Rains). The annual abscission of leaves is another way of "preventing" an excessive buildup of solutes in the cell wall water, as well as returning the mineral nutrients to the soil (see Epstein).

Resistances and areas. We will now consider the resistances to water flow in those parts of the soil-plant-atmosphere continuum where water moves as a liquid. (We have already considered the gaseous parts of the pathway in Ch. 7.) If we let the flux of water equal the drop in water potential across some component divided by its resistance, we would have only part of the story, since we should also consider the relative areas of each component as well as whether $\Delta\Psi$ does indeed represent the driving force. Also, $\Delta\Psi$ represents the relative energies of water only at constant

T , and thus we should compare water potentials ideally only between locations at the same temperature. The root and adjacent soil are usually at the same temperature, as are the leaf mesophyll cells and air in the intercellular air spaces. However, roots and leaves generally are *not* at the same temperature. Nevertheless, the mechanical criterion of the pressure gradient (Eq. 8.9) turns out to be sufficiently accurate for describing the flow in the xylem or the phloem, even when a temperature difference exists along the pathway (see Spanner).

Let us designate the average volume flux of water across area A^j of component j by $J_{V_w}^j$. A^j could be the root surface area, the effective cross-sectional area of the xylem, or the area of one side of the leaves. In the steady state, the product $J_{V_w}^j A^j$ is essentially constant, since nearly all of the water taken up by the root is lost by transpiration, and thus the same volume of water moves across each component along the pathway per unit time. We will represent the drop in water potential across component j by $\Delta\Psi^j$, and define the resistance of component j (R^j) as follows:

$$J_{V_w}^j A^j = \frac{\Delta\Psi^j}{R^j} \cong \text{constant} \quad (8.10)$$

where $J_{V_w}^j A^j$ is the volume of water crossing component j in unit time.

A relation similar to Equation 8.10 was proposed by T. H. van den Honert in 1948; both can be used to describe certain overall characteristics of water flow in the soil-plant-atmosphere system. However, $\Delta\Psi^j$ does not always represent the driving force on water, e.g., a change in the osmotic pressure component of Ψ has no direct effect on the flow in the xylem or the phloem. Also, such a relation is not useful for a gas phase, since the value of the resistance R^j depends on the concentration of water vapor (see p. 313).

In the last chapter we indicated that J_{wv} for transpiration by an exposed leaf of a mesophyte might be $7.6 \mu\text{g of water}/\text{cm}^2\text{-sec}$ (p. 323), while in this chapter we calculated that J_V into a certain length of a *young* root can be $1.1 \times 10^{-5} \text{ cm}/\text{sec}$. Nearly all of the volume flux into a root is due to water, so that J_{V_w} for this root is also about $1.1 \times 10^{-5} \text{ cm}/\text{sec}$, which corresponds to $(1.1 \times 10^{-5} \text{ cm}/\text{sec})(1.00 \text{ g of water}/\text{cm}^3)$, or $11 \mu\text{g}/\text{cm}^2\text{-sec}$. For a plant having such leaves and roots, Equation 8.10 indicates that in order for the amount of water flowing across each component to be the same, the area (of one side) of the leaves must be $(11 \mu\text{g}/\text{cm}^2\text{-sec})/(7.6 \mu\text{g}/\text{cm}^2\text{-sec})$, or 1.4 times larger than the surface area of the roots. We should note, however, that the root system of many plants is quite extensive, and so A^{root} can be 20–100 times larger than the surface area of the leaves. Also, $J_{V_w}^{\text{root}}$ depends markedly on root age and hence varies along the length of a root, e.g., it

is usually considerably lower for older roots (see Epstein, and Slatyer). J_v can be 0.1 cm/sec in the xylem (p. 393). Again using Equation 8.10, we conclude that the surface area of the roots would then be $(0.1 \text{ cm/sec}) / (1.1 \times 10^{-5} \text{ cm/sec})$, or 10^4 times larger than the cross-sectional area of the conducting parts of the xylem. Very few measurements of A^j and $J_{v_w}^j$ have actually been made for the sequential components involved in water flow in a plant.

For the example in Table 8.2, the drop in water potential is 2 bars across the soil part of the pathway, 1 bar from the root surface to the root xylem, and 2 bars along the xylem, which suggests the relative magnitudes of the three resistances involved ($\Delta\Psi^j \cong \text{constant} \times R^j$, Eq. 8.10). As the soil dries out, the relative size of the water potential drop in the soil usually becomes larger (cf. p. 389). Also, the rapid uptake of soil water by the root during those parts of the day when transpiration is particularly high can lead to a large hydrostatic pressure gradient in Darcy's law (Eq. 8.7), and $\Delta\Psi^{\text{soil}}$ from the bulk soil to the root then increases. In fact, R^{soil} is often the largest resistance for that part of the soil-plant-atmosphere system where water moves predominantly as a liquid (water can also move as a vapor in the soil, see p. 388). The resistance of the root epidermis, cortex, and endodermis is generally somewhat less than R^{soil} . The resistance of the xylem depends on its length, since the drop in water potential along the xylem is often about 0.2 bar/m (cf. p. 393).

When $J_{v_w}^j$ is in cm/sec, A^j in cm^2 , and $\Delta\Psi^j$ in bars, Equation 8.10 indicates that the units of R^j are bar-sec/ cm^3 . For young sunflower and tomato plants, the resistance from the root surface to the leaf mesophyll cells (R^{plant}) is about 1000 bar-sec/ cm^3 when the plants are approximately 30 cm tall and $\Delta\Psi^{\text{plant}}$ is close to 2 bars (see Kramer). By Equation 8.10, $J_{v_w}^j A^j$ for each component would then be $(2 \text{ bars}) / (1000 \text{ bar-sec/cm}^3)$, or $2 \times 10^{-3} \text{ cm}^3$ of water/sec.

Diurnal changes. We have already indicated certain diurnal changes that take place in the soil-plant-atmosphere system. For instance, the soil temperature changes diurnally, which in turn affects the CO_2 evolution by respiration in soil microorganisms as well as in root cells. Also, the hydrostatic pressure in the xylem is more negative when the rate of transpiration is high, causing plants to decrease slightly in diameter during the day. All such diurnal changes are direct or indirect consequences of the daily variation in sunlight. Of course, the rate of photosynthesis also changes during the day. Photosynthesis not only depends on the illumination level but is also affected by the leaf temperature, which depends on the diurnally varying net radiation balance for a leaf.

Marked diurnal changes also occur in the water potentials in the soil-plant-atmosphere system. Let us consider a plant in a well-watered soil (Fig. 8.6). At night transpiration essentially ceases, since the stomates then close; the water potentials in the soil, the root, and a leaf may then all become nearly equal (and close to zero bars). As the light part of a day begins, the stomates open. Transpiration then removes water from the leaf and Ψ^{leaf} decreases (Fig. 8.6). After a short lag, Ψ^{root} begins to decrease, but only to about -3 bars, since plenty of water is initially available in the wet soil. At dusk, these changes in Ψ^{leaf} and Ψ^{root} are reversed. As soil moisture is lost, Ψ^{soil} becomes more negative day-by-day, so that Ψ^{root} and Ψ^{leaf} also become more negative. (We are assuming that Ψ^{air} here remains essentially unchanged and is much lower than the other water potentials. Moreover, we are also assuming isothermal conditions, which is unrealistic, but, fortunately, will give us a useful approximation.) As the soil becomes drier, a steeper gradient in water potential is necessary to sustain the water flow up to the root, and therefore the difference between Ψ^{soil} and Ψ^{root} becomes

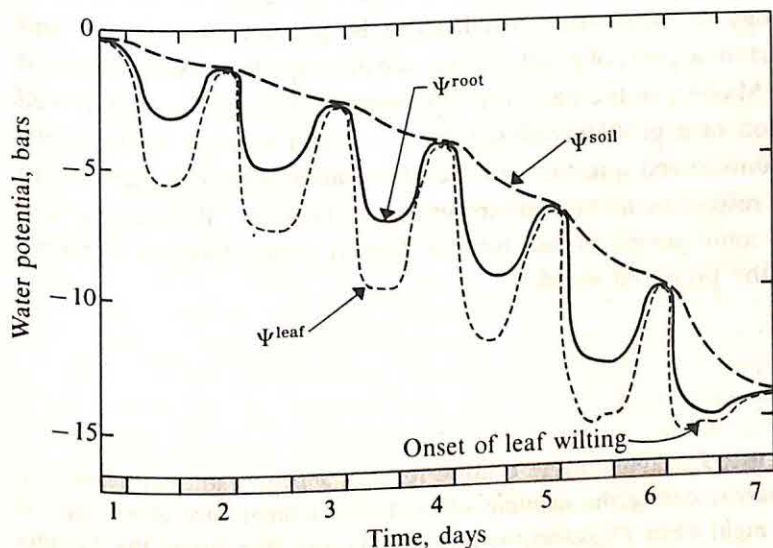


Figure 8.6

Schematic representation of daily changes in the water potentials in the soil, root, and leaf of a plant in an initially wet soil that dries out over a one week period. Ψ^{soil} is the water potential in the bulk soil, Ψ^{root} is that in the root xylem, and Ψ^{leaf} is the value in a leaf mesophyll cell. (Adapted by permission from R. O. Slatyer, *Plant-Water Relationships*, Academic Press, New York, 1967, p. 276.)

larger day-by-day (Fig. 8.6). As Ψ^{leaf} becomes more negative on ensuing days, the leaf turgor pressure decreases, and eventually the hydrostatic pressure in the mesophyll cells of the leaf becomes zero some time during the day, e.g., when Ψ^{leaf} becomes -15 bars in response to the decreasing Ψ^{soil} . The leaf thus wilts, but recovers at night. Permanent wilting and damage to the leaf may result when Ψ^{soil} becomes lower on subsequent days. This portrayal of successive diurnal changes of Ψ^{soil} , Ψ^{root} , and Ψ^{leaf} illustrates that even such nonequilibrium processes in the soil-plant-air system can be analyzed in terms of the water potential.

What new applications of physics and chemistry might we expect in plant physiology in the future? The quantitative approach of the last two chapters should be expanded to include the interdependence of forces and fluxes as presented by irreversible thermodynamics. Cross-coefficients relating fluxes of charge to those of neutral species such as water need further consideration. Nonisothermal conditions must also be adequately handled. Of even greater potential impact is the application of a mathematical approach to the very complex field of plant growth and development. Hormone action, differentiation, photomorphogenesis, reproduction, and senescence are all areas of plant physiology for which new insights can be provided by physics and chemistry. Certain aspects of plant ecology are also ripe for physicochemical explanations. Mastery of the basic principles emphasized in this text as well as the adoption of a problem-solving approach will help us deal quantitatively with unresolved questions in the above areas and also allow us to predict plant responses to new environmental situations. Progress in any field requires some good fortune. But, as Pasteur would have it, "Chance favours only the prepared mind."

Problems

8.1 Suppose that J_{wv} above some canopy reaches a peak value equivalent to 1.0 mm of water/hr during the daytime when the air temperature is 30° , and is 0.10 mm/hr at night when T^{ta} corresponds to 20° . Assume that during the daytime the relative humidity decreases by 20% across the first 30 m of turbulent air and that the eddy diffusion coefficient halves at night because of an ambient wind velocity lower than during the daytime. (a) What are J_{wv} in $\mu\text{moles}/\text{cm}^2\text{-sec}$ and $\Delta c_{wv}^{\text{ta}}$ in $\mu\text{moles}/\text{cm}^3$ during peak transpiration? (b) What is R_{wv}^{ta} (over the first 30 m) during peak transpiration and at night? (c) What is $\Delta c_{wv}^{\text{ta}}$ at night in $\mu\text{moles}/\text{cm}^3$? To what drop in relative humidity does this correspond? (d) If J_{wv}/J_{CO_2} is -700 during peak transpiration, what are J_{CO_2} and $\Delta c_{\text{CO}_2}^{\text{ta}}$ then? (e) What is

K_{CO_2} for the first 30 m of turbulent air at night? (f) How long would it take water vapor to diffuse 1 m at night by eddy diffusion and by ordinary diffusion? Assume that Equation 1.6 ($x_e^2 = 4D_j t$) applies, where D_{wv} is $0.25 \text{ cm}^2/\text{sec}$ at 20° , and K_{wv} has the value averaged over the first 30 m of turbulent air.

8.2 Let us suppose that the foliar absorption coefficient is 0.7 for trees with an average leaf area index of 8.0. (a) If the light compensation point for CO_2 fixation is 400 lux, what are the cumulative leaf areas for light compensation when 100,000 lux, 10,000 lux, 1000 lux, and 0 lux are incident on the canopy? (b) Suppose that $J_{\text{wv}}/J_{\text{CO}_2}$ for the soil is 200, that 90% of the water vapor passing out of the canopy comes from the leaves, and that the net photosynthetic rate for the forest (using CO_2 from above the canopy as well as from the soil) corresponds to 20 kg of carbohydrate/hectare-hr. If J_{wv} from the soil is $0.06 \mu\text{mole}/\text{cm}^2\text{-sec}$, what are the J_{CO_2} s up from the soil and down into the canopy? (c) What is the absolute value of $J_{\text{wv}}/J_{\text{CO}_2}$ above the canopy under the conditions of (b)? (d) When the leaves are randomly distributed with respect to distance above the ground and the trees are 16 m tall, at what level does the maximum upward flux of CO_2 occur when 10,000 lux is incident on the canopy? (e) If the net rate of photosynthesis is proportional to illumination level, about where does $c_{\text{CO}_2}^{\text{ta}}$ achieve a minimum? Assume that the conditions are as in (b) and (d) and that the maximum upward J_{CO_2} is $0.5 \text{ nmole}/\text{cm}^2\text{-sec}$. Note that in the present case the illumination halves every 2 m downward in the vegetation.

8.3 The global radiation incident on the ground below some vegetation averages $0.15 \text{ cal}/\text{cm}^2\text{-min}$. We will assume that the bulk of the vegetation is at 22° , the top of the soil is at 20° , and that both emit like ideal blackbodies. (a) What is the net radiation balance for the soil? (b) Suppose that there are 4 plants/ m^2 of ground and that their average stem diameters are 3 cm. If the thermal conductivity coefficient of the stem is the same as that of water, and the temperature drops from that of the ground to that of the bulk of the vegetation in 0.8 m, what is the rate of heat conduction in cal/min down each stem? What is the average value of such J_H^c per cm^2 of the ground? (c) Suppose that the 4 mm of air immediately above the ground acts like an unstirred layer and that the air temperature at 0.4 cm is 21° . What is the rate of heat conduction from the soil into the air? (d) What is J_H^c into the soil in the steady state if $0.03 \mu\text{mole}$ of water/ $\text{cm}^2\text{-sec}$ evaporates from the upper part of the soil where the radiation is absorbed? (e) If K^{soil} is the same as K^{water} , what is $\partial T/\partial z$ in the upper part of the soil?

8.4 A horizontal xylem element has a cross-sectional area of 0.004 mm^2 and conducts water at 20° at a rate of $0.02 \text{ ml}/\text{hr}$. (a) What is the mean velocity of the fluid in the xylem element? (b) What pressure gradient is necessary to cause such a flow? (c) If the pressure gradient remained the same, but cell walls with interstices 20 nm across filled the xylem element, what would be the mean velocity of fluid movement? Assume that the entire area of the cell walls is available for conduction.

8.5 A horizontal phloem vessel has an effective radius of $10\text{ }\mu\text{m}$. Assume that D_{sucrose} in the phloem solution, which has a viscosity of 0.015 poise, is $0.3 \times 10^{-5}\text{ cm}^2/\text{sec}$ at 20° . (a) If there is no flow in the vessel and a thin layer of ^{14}C -sucrose is inserted, how long would it take by diffusion for the radioactive label at 1 cm and at 1 m to be 37% of the value at the plane of insertion? (b) If there is a pressure gradient of -0.20 bar/m which leads to Poiseuille flow in the phloem, what is J_v there? (c) For the conditions of (b), how long does it take on the average for sucrose to move 1 cm and 1 m in the phloem vessel (ignore concomitant diffusion)? Compare your values with answers to (a). (d) For the pressure gradient in (b), what would be J_v in a vertical phloem vessel?

8.6 Suppose that L_{soil} is $10^{-2}\text{ cm}^2/\text{sec-bar}$ and $2 \times 10^{-7}\text{ cm}^2/\text{sec-bar}$ when Ψ_{soil} is -0.1 bar and -14 bars , respectively. For a plant in the wet soil, J_v^{xylem} is 0.2 cm/sec and Ψ^{leaf} is -2 bars for a leaf 3 m above the ground. We will assume that the roots are 3 mm in diameter and that their surface area is 5 times that of one side of the leaves and 10^5 times larger than the conducting area of the xylem. Assume that all temperatures are 20° and ignore all osmotic pressures. (a) What are the radii of curvature at hemispherical air-liquid interfaces in the wet and the dry soils? (b) If water moves a distance of 0.8 cm to reach the root, what is the drop in hydrostatic pressure over that interval in the wet soil? (c) Suppose that the root xylem is arranged concentrically in a ring $500\text{ }\mu\text{m}$ below the root surface. If the average conductivity of the epidermis, cortex, endodermis, and xylem cell walls in the root is like that of the dry soil, what is the drop in hydrostatic pressure across them? (d) If the water in the leaf xylem is in equilibrium with that in the vacuoles of mesophyll cells, what is the average hydrostatic pressure gradient in the xylem for the wet soil case (assume that A^{xylem} is constant throughout the plant)? (e) If A^{mes}/A is 20, and the cell wall pores are 10 nm in diameter and $1\text{ }\mu\text{m}$ long, what ΔP (in bars) along them will account for the rate of transpiration for the plant in the wet soil? (f) If the relative humidity above the dry soil is increased to 99%, what is J_v^{xylem} then?

References

- Briggs, G. E. 1967. *Movement of Water in Plants*, Blackwell Scientific Publications, Oxford.
- Canny, M. J. 1971. Translocation: Mechanisms and kinetics. *Annual Review of Plant Physiology* **22**: 237-260.
- Childs, E. C. 1969. *The Physical Basis of Soil Water Phenomena*, Wiley, New York.
- Cowan, I. R. 1968. Mass, heat and momentum exchange between stands of plants and their atmospheric environment. *Quarterly Journal of the Royal Meteorological Society* **94**: 523-544.
- Crafts, A. S., and C. E. Crisp. 1971. *Phloem Transport in Plants*, W. H. Freeman and Company, San Francisco.

- Eastin, J. D., F. A. Haskins, C. Y. Sullivan, and C. H. M. van Bavel (eds.). 1969. *Physiological Aspects of Crop Yield*, American Society of Agronomy, Madison.
- Epstein, E. 1972. *Mineral Nutrition of Plants: Principles and Perspectives*, Wiley, New York.
- Esau, K. 1969. *The Phloem*, *Encyclopedia of Plant Anatomy*, 2nd ed., Vol. 5, Part 2, Gebrüder Borntraeger, Berlin.
- Evans, L. T. (ed.). 1963. *Environmental Control of Plant Growth*, Academic Press, New York.
- Hillel, D. 1971. *Soil and Water—Physical Principles and Processes*, Academic Press, New York.
- Jordan, W. R., and J. T. Ritchie. 1971. Influence of soil water stress on evaporation, root absorption, and internal water status of cotton. *Plant Physiology* **48**: 783–788.
- Kozlowski, T. T. (ed.). 1968. *Water Deficits and Plant Growth*, Vols. I and II, Academic Press, New York.
- Kozlowski, T. T. (ed.). 1972. *Water Deficits and Plant Growth*, Vol. III, Academic Press, New York.
- Kramer, P. J. 1969. *Plant and Soil Water Relationships: A Modern Synthesis*, McGraw-Hill, New York.
- Lemon, E., D. W. Stewart, and R. W. Shawcroft. 1971. The sun's work in a cornfield. *Science* **174**: 371–378.
- Loomis, R. S., W. A. Williams, and A. E. Hall. 1971. Agricultural productivity. *Annual Review of Plant Physiology* **22**: 431–468.
- Lüttge, U. 1971. Structure and function of plant glands. *Annual Review of Plant Physiology* **22**: 23–44.
- Millington, R. J., and D. B. Peters. 1969. Exchange (mass transfer) coefficients in crop canopies. *Agronomy Journal* **61**: 815–819.
- Moss, D. N., and R. B. Musgrave. 1971. Photosynthesis and crop production. *Advances in Agronomy* **23**: 317–336.
- Oliver, H. R. 1971. Wind profiles in and above a forest canopy. *Quarterly Journal of the Royal Meteorological Society* **97**: 548–553.
- Paltridge, G. W. 1972. Experiments on a mathematical model of a pasture. *Agricultural Meteorology* **10**: 39–54.
- Rains, D. W. 1972. Salt transport by plants in relation to salinity. *Annual Review of Plant Physiology* **23**: 367–388.
- Rose, C. W. 1966. *Agricultural Physics*, Pergamon, Oxford.
- San Pietro, A., F. A. Greer, and T. J. Army (eds.). 1967. *Harvesting the Sun: Photosynthesis in Plant Life*, Academic Press, New York.
- Šesták, Z., J. Čatský, and P. G. Jarvis (eds.). 1971. *Plant Photosynthetic Production: Manual of Methods*, Junk, The Hague.
- Šetlik, I. (ed.). 1970. *Prediction and Measurement of Photosynthetic Productivity*, Pudoc, Centre for Agricultural Publishing and Documentation, Wageningen (the Netherlands).

- Slatyer, R. O. 1967. *Plant-Water Relationships*, Academic Press, New York.
- Spanner, D. C. 1972. Plants, water, and some other topics, in *Psychrometry in Water Relations Research*, R. W. Brown and B. P. VanHaveren, eds., Utah Agricultural Experiment Station, Utah State University, Logan, in press.
- Streeter, V. L. 1966. *Fluid Mechanics*, 4th ed., McGraw-Hill, New York.
- Taylor, S. A., and G. L. Ashcroft. 1972. *Physical Edaphology*, W. H. Freeman and Company, San Francisco.
- van Wijk, W. R. (ed.). 1966. *Physics of Plant Environment*, 2nd ed., North-Holland, Amsterdam.
- World Meteorological Organization. 1968. *Agricultural Meteorology: Proceedings of the WHO Seminar, Melbourne, Australia, 28 November-16 December 1966*, Vols. 1 and 2, Bureau of Meteorology, Melbourne.
- Yoshida, S. 1972. Physiological aspects of grain yield. *Annual Review of Plant Physiology* 23: 437-464.
- Zelitch, I. 1971. *Photosynthesis, Photorespiration, and Plant Productivity*, Academic Press, New York.
- Zimmermann, M. H., and C. L. Brown. 1971. *Trees—Structure and Function*, Springer Verlag, New York.

APPENDICES

FREQUENTLY USED VARIABLES AND THEIR UNITS

<i>Symbol</i>	<i>Description</i>	<i>Typical units</i>
a	absorptivity	dimensionless
a^{st}	mean area of stomate	cm^2
a_{IR}	absorptivity in infrared region	dimensionless
a_j	activity of species j	same as concentration*
b	nonosmotic volume	μm^3
b	optical path length	cm
c_j	concentration of species j	moles/liter
$\bar{c}_s$	a mean concentration of solute s	moles/cm^3
d	depth or distance	cm
e_{IR}	emissivity in infrared region	dimensionless
h	height	cm
i	electrical current	amperes
k	foliar absorption coefficient	dimensionless
k_j	first-order rate constant for the j^{th} process	sec^{-1}
l	length	cm
m_j	mass/mole of species j (molecular weight)	g/mole
n	number of stomates/unit area	$\text{number}/\text{cm}^2$
$n(E)$	number of molecules with energy of E or greater	moles
n_j	amount of species j	moles
q	number of electrons transferred/molecule	dimensionless
r	radius	cm

(continued)

Symbol	Description	Typical units
r	reflectivity	dimensionless
s_j	amount of species j	moles
t	time	sec
u_j	mobility of species j	velocity per unit force
v	velocity of light	m/sec
v^{wind}	wind velocity	cm/sec
v_j	velocity of species j	cm/sec
v_{CO_2}	rate of photosynthesis/unit volume	$\mu\text{moles/cm}^3\text{-sec}$
x	distance	cm
z	altitude	cm
z_j	charge number of ionic species j	dimensionless
A	area	cm^2
A	absorbance (also called "optical density")	dimensionless
C	capacitance	farads
C'	capacitance/unit area	$\mu\text{f/cm}^2$
D	dielectric constant	dimensionless
D_j	diffusion coefficient of species j	cm^2/sec
E	light energy	ev
E	kinetic energy	ev
E	electrical potential	mv
E_j	redox potential of species j	mv
$E_j^{*,\text{H}}$	midpoint redox potential of species j referred to standard hydrogen electrode	mv
E_M	electrical potential difference across a membrane	mv
E_{N_j}	Nernst potential of species j	mv
F	average cumulative leaf area/ground area	dimensionless
G	Gibbs free energy	kcal
G/n_j	Gibbs free energy/mole of some product or reactant	kcal/mole
I	electrical current	amperes
I	flux of electromagnetic radiation	$\text{photons/cm}^2\text{-sec}$
J_j	flux of moles of species j	$\text{moles/cm}^2\text{-sec}$
J_j^{in}	influx of species j	$\text{moles/cm}^2\text{-sec}$

<i>Symbol</i>	<i>Description</i>	<i>Typical units</i>
J_j^{out}	efflux of species j	moles/cm ² -sec
J_{V_j}	volume flux of species j	cm/sec (i.e., cm ³ /cm ² -sec)
J_V	total volume flux	cm/sec
K^j	thermal conductivity coefficient of region j	(cal/cm ² -min)/(°/cm)
K_j	partition coefficient of species j	dimensionless
K_j	concentration for half-maximal uptake of species j	M
K_j	eddy diffusion coefficient of gaseous species j	cm ² /sec
K	equilibrium constant	molarity raised to some power
$K_{\text{pH } 7}$	equilibrium constant at pH 7	molarity raised to some power
L	length	cm
L^{soil}	hydraulic conductivity coefficient of the soil	cm ² /sec-bar
L_{jk}	Onsager or phenomenological coefficient	flux per unit force
L_P	hydraulic conductivity coefficient (in irreversible thermodynamics)	cm/sec-bar
L_w	water conductivity coefficient	cm/sec-bar
M_j	amount of species j /unit area	moles/cm ²
N_j	mole fraction of species j	dimensionless
P	hydrostatic pressure	bars
P_j	permeability coefficient of species j	cm/sec
P_j	partial pressure of gaseous species j	bars
Q	charge	coulombs
Q_{10}	temperature coefficient	dimensionless
R	electrical resistance	ohms
R^j	resistance of component j across which water moves as a liquid	bar-sec/cm ³
R_j	resistance for gaseous species j	sec/cm
S	magnitude of net spin	dimensionless
S	total flux of solar radiation, i.e., global radiation	cal/cm ² -min

(continued)

<i>Symbol</i>	<i>Description</i>	<i>Typical units</i>
T	temperature	° Kelvin
U	kinetic energy	ev/molecule
U_B	minimum kinetic energy to cross barrier	ev/molecule
V	volume	cm ³
$\bar{V}_j$	partial molal volume of species j	cm ³ /mole
α	contact angle	°
γ_j	activity coefficient of species j	dimensionless*
$\gamma_{\pm}$	mean activity coefficient of cation-anion pair	dimensionless
δ^a	thickness of unstirred air layer	cm
ϵ_{λ}	absorption coefficient at wavelength λ	liters/mole-cm
η	viscosity	poise (dyne-sec/cm ²)
λ	wavelength of light	nm
μ_j	chemical potential of species j	cal/mole
ν	frequency of electromagnetic radiation	cycles/sec (Hertz)
π	total osmotic pressure	bars
π_j	osmotic pressure of species j	bars
π_s	osmotic pressure due to solutes	bars
ρ	density	g/cm ³
σ	surface tension	dynes/cm
σ	reflection coefficient	dimensionless
σ_j	reflection coefficient of species j	dimensionless
σ_L	longitudinal stress	bars
σ_T	tangential stress	bars
τ	matric pressure or potential	bars
τ	lifetime	sec
τ_j	lifetime for the j^{th} de-excitation process	sec
φ_j	osmotic coefficient of species j	dimensionless
Φ_i	quantum yield or efficiency for i^{th} de-excitation pathway	dimensionless
Ψ	water potential	bars

* The activity, a_j , is often considered to be dimensionless, in which case the activity coefficient, γ_j , has the units of reciprocal concentration ($a_j = \gamma_j c_j$, Eq. 2.5).

Symbol	Description	Magnitude
	solar constant	2.00 cal/cm ² -min 1.39 × 10 ⁵ ergs/cm ² -sec 0.139 watt/cm ²
D_{CO_2}	diffusion coefficient of carbon dioxide	0.16 cm ² /sec in air at 20°
D_{O_2}	diffusion coefficient of oxygen	0.20 cm ² /sec in air at 20°
D_{wv}	diffusion coefficient of water vapor	0.25 cm ² /sec in air at 20°
K^{air}	thermal conductivity coefficient of dry air	3.25 × 10 ⁻³ (cal/cm ² -min)/(°/cm) at 0° 3.31 " 10° 3.37 " 20° 3.43 " 30° 3.50 " 40° 3.56 " 50° 3.62 " 60°
K^{water}	thermal conductivity coefficient of water	0.081 (cal/cm ² -min)/(°/cm) at 0° 0.086 " 20° 0.090 " 40°
c_{wv}^*	saturation concentration of water vapor (i.e., at 100% relative humidity)/unit volume of air	2.36 μg/cm ³ (0.131 μmole/cm ³) at -10° 3.40 " 0.189 " -5° 4.85 " 0.269 " 0° 6.80 " 0.378 " 5° 9.41 " 0.522 " 10° 12.8 " 0.712 " 15° 17.3 " 0.960 " 20° 23.0 " 1.28 " 25° 30.4 " 1.69 " 30° 51.1 " 2.84 " 40° 83.2 " 4.62 " 50°
H_{sub}	heat of sublimation of water	12.27 kcal/mole (680 cal/g) at -10° 12.22 " 677 " -5° 12.18 " 675 " 0°
H_{vap}	heat of vaporization of water	10.73 kcal/mole (595 cal/g) at 0° 10.63 " 590 " 10° 10.54 " 585 " 20° 10.49 " 582 " 25° 10.44 " 580 " 30° 10.34 " 574 " 40° 10.24 " 568 " 50° 9.71 " 539 " 100°

FREQUENTLY USED CONSTANTS AND THEIR NUMERICAL VALUES

<i>Symbol</i>	<i>Description</i>	<i>Magnitude</i>
	speed of light in vacuum	2.998×10^8 m/sec
	base for natural logarithm	2.71828 ($1/e = 0.368$)
	gravitational acceleration	980.6 cm/sec ² (sea level,* 45° latitude)
		978.0 " 0° "
		983.2 " 90° "
	Planck's constant	6.6256×10^{-27} erg-sec
		0.4136×10^{-14} ev-sec
	Boltzmann's constant	1.584×10^{-37} kcal-sec
		1.381×10^{-16} erg/molecule-°K
		8.617×10^{-5} ev/molecule-°K
	Faraday	0.693
		96,487 coulombs/mole
		96,487 joules/mole-v
		23,060 cal/mole-v
		23.06 kcal/mole-v
	Avogadro's number	0.02306 kcal/mole-mv
	gas constant	6.02252×10^{23} molecules/mole
		8.3143 joules/mole-°K
		1.987 cal/mole-°K
		0.082054 liter-atmosphere/mole-°K
		0.083141 liter-bar/mole-°K
		83.141 cm ³ -bar/mole-°K
	permittivity of a vacuum**	8.85415×10^{-12} coulomb ² /newton-m ²
		8.85415×10^{-12} coulomb/v-m
	Stefan-Boltzmann constant	8.130×10^{-11} cal cm ⁻² min ⁻¹ °K ⁻⁴
		5.670×10^{-5} erg cm ⁻² sec ⁻¹ °K ⁻⁴
		5.670×10^{-12} watt cm ⁻² °K ⁻⁴

(continued)

<i>Symbol</i>	<i>Description</i>	<i>Magnitude</i>
P_{wv}^*	saturation vapor pressure of water	6.1 mbars (4.6 mm Hg) at 0°
		12.3 " 9.2 " 10°
		23.3 " 17.5 " 20°
		31.7 " 23.8 " 25°
		42.4 " 31.8 " 30°
		73.7 " 55.3 " 40°
		123 " 92.5 " 50°
$\bar{V}_w$	partial molal volume of water	18.048 cm ³ /mole at 20°
η_w	viscosity of water	0.01787 poise (dyne-sec/cm ²) at 0°
		0.01306 poise at 10°
		0.01002 " 20°
		0.00798 " 30°
		0.00653 " 40°
ρ_w	density of water	0.9999 g/cm ³ at 0°
		1.0000 " 4°
		0.9997 " 10°
		0.9982 " 20°
		0.9957 " 30°
		0.9923 " 40°
σ_w	surface tension of water	75.6 dynes/cm at 0°
		74.2 " 10°
		72.8 " 20°
		71.2 " 30°
		69.6 " 40°
		7.18 × 10 ⁻⁵ atmosphere-cm at 20°
		7.28 × 10 ⁻⁵ bar-cm at 20°

* The correction for height above sea level is -0.000309 cm/sec² per meter of altitude.

** The so-called rationalized meter-kilogram-second (mks) system has been adopted for expressing electrical phenomena in this text (cf. Eq. 2.3). "Rationalized" means that the factors 2π or 4π appear in equations where they might reasonably be expected based on the geometry, e.g., in cases involving cylindrical or spherical symmetry, respectively. (In a nonrationalized system, π occurs in equations describing situations without cylindrical or spherical symmetry.) The numerical value of certain constants such as the permittivity ϵ_0 depends on whether a rationalized or a nonrationalized system of equations is used. In the mks system, charges are expressed in coulombs, which becomes a fundamental "dimension" like meter or kilogram. Since an ampere is a coulomb/sec, the mks system is often called an mksa system (*a* for ampere) to emphasize this convention of treating electrical units. In the frequently used nonrationalized centimeter-gram-second (cgs) system, no special unit is picked for charge, which must then be expressed in so-called electrostatic units, while cgs potentials are in units 299.80 times larger than ordinary volts. Amperes and ohms, which are electrical units commonly used in the laboratory, refer to the mks (mksa) system.

CONVERSION FACTORS

<i>Quantity</i>	<i>Equals</i>
acre	4047 m ²
ampere	0.4047 hectare 1 coulomb/sec
Ångstrom	1 volt/ohm 0.1 nm 10 ⁻⁸ cm 10 ⁻⁴ μm 10 ⁻¹⁰ m
atmosphere	1.013 × 10 ⁶ dynes/cm ² 0.1013 joule/cm ³ 1.013 bars 0.0242 cal/cm ³ 1.033 kg/cm ² (at sea level)*
bar	76.0 cm or 760 mm Hg (at sea level) 10 ⁶ dynes/cm ² 0.1 joule/cm ³ 0.987 atmosphere 0.0239 cal/cm ³ 1.020 kg/cm ² (at sea level)*
calorie	75.0 cm or 750 mm Hg (at sea level) 4.184 joules
calorie/cm ²	4.184 × 10 ⁷ ergs 1 langley

<i>Quantity</i>	<i>Equals</i>
calorie/cm ³	41.84 bars
calorie/min	0.0697 joule/sec 0.0697 watt
calorie/sec	4.184 joules/sec 4.184 watts
coulomb	1 joule/v
coulomb-volt	1 joule
day	86,400 sec
dyne	1 erg/cm 1 g-cm/sec ² 10 ⁻⁵ newton
dyne-cm	1 erg
dyne/cm ²	10 ⁻⁶ bar
Einstein	6.023 × 10 ²³ photons
electron volt	1.602 × 10 ⁻¹² erg
electron volt/molecule	23.06 kcal/mole
erg	1 dyne-cm 1 g-cm ² /sec ² 10 ⁻⁷ joule 6.242 × 10 ¹¹ ev 2.390 × 10 ⁻⁸ cal 2.390 × 10 ⁻¹¹ kcal
farad	1 coulomb/v
footcandle	10.76 lux
gram	1 dyne-sec ² /cm
<i>hc</i>	1240 ev-nm
hectare	10 ⁴ m ² 2.47 acres
Hertz	1 cycle/sec
hour	3600 sec
joule	1 newton-m 1 coulomb-v 10 ⁷ ergs 0.239 cal 10 cm ³ -bar
joule/sec	watt

(continued)

<i>Quantity</i>	<i>Equals</i>
kilocalorie	4.184×10^3 joules
	4.184×10^{10} ergs
kilocalorie/mole	0.0434 ev/molecule
kilometer/hr	27.78 cm/sec
	0.6214 mile/hr
kilowatt-hr	3.60×10^6 joules
	8.60×10^5 cal
kT	0.0253 ev/molecule at 20°
	0.0257 " 25°
	0.0261 " 30°
langley	1 cal/cm ²
liter-atmosphere	24.2 cal
liter-bar	23.9 cal
	100 joules
ln	2.303 log
lux	1 lumen/m ²
m/hr	0.02778 cm/sec
micron (μ m)	10^4 Ångstroms
	10^{-3} mm
	10^{-4} cm
mile/hr	44.70 cm/sec
	1.609 km/hr
millibar	10^{-3} bar
	10^3 dynes/cm ²
	0.987×10^{-3} atmosphere
newton	1 kg-m/sec ²
	10^5 dynes
newton-m	1 joule
Nhc	28.60 kcal- μ m/mole
	28.600 kcal-nm/mole
Nk	R
poise	dyne-sec/cm ²
RT	543 cal/mole at 0°
	582 " 20°
	592 " 25°
	2271 joules/mole at 0°
	2437 " 20°
	2479 " 25°

Quantity	Equals
	22.41 liter-atmospheres/mole at 0°
	24.05 " 20°
	24.46 " 25°
	22.71 liter-bars/mole at 0°
	24.37 " 20°
	24.79 " 25°
	22,710 cm ³ -bars/mole at 0°
	24,370 " 20°
	24,790 " 25°
2.303 RT	1.342 kcal/mole at 20°
	1.364 kcal/mole at 25°
	56,120 cm ³ -bars/mole at 20°
RT/F	25.3 mv at 20°
	25.7 mv at 25°
2.303 RT/F	58.2 mv at 20°
	59.2 " 25°
	60.2 " 30°
RT/ $\bar{V}_w$	32.4 cal/cm ³ at 20°
	135.0 joules/cm ³ at 20°
	1330 atmospheres at 20°
	1350 bars at 20°
	1373 bars at 25°
2.303 RT/ $\bar{V}_w$	3063 atmospheres at 20°
	3109 bars at 20°
	3162 bars at 25°
$\rho_w g$	0.0966 atmosphere/m (20° centigrade, sea level, 45° latitude)
	0.0979 bar/m (20° centigrade, sea level, 45° latitude)
	979 dynes/cm ³ (20° centigrade, sea level, 45° latitude)
torr	1 mm Hg
	0.001333 bar (at sea level)
	1333 dynes/cm ² (at sea level)
watt	1 joule/sec
	10 ⁷ ergs/sec

(continued)

*Quantity**Equals*

year	3.16×10^7 sec
	5.256×10^5 min
	8760 hr

* Often it proves convenient to express forces in units which really refer to mass. For instance, the "force"/unit area used with Young's modulus (Eq. 1.13) is often given in kg/cm^2 . Moreover, a bar, which is 10^6 dynes of force per cm^2 , is often referred to as $1.020 \text{ kg}/\text{cm}^2$ at sea level, where kilogram is obviously a mass unit. To see why this is possible, consider the force F exerted by gravity on a body of mass m . This force is equal to mg , g being the gravitational acceleration. (See Appendix II.) Thus F/g can be used to represent a force, but the units are those of mass. Referring to the above example, a bar is $10^6 \text{ dynes}/\text{cm}^2$, which is equivalent to $(10^6 \text{ dynes}/\text{cm}^2)/(980 \text{ cm}/\text{sec}^2)$ when a gravitational acceleration of $980 \text{ cm}/\text{sec}^2$ is used. Since $(\text{dynes})/(\text{cm}/\text{sec}^2)$ are simply grams, a bar is equivalent at sea level to $(10^6)/(980) \text{ g}/\text{cm}^2$ which is $(10^3)/(980) \text{ kg}/\text{cm}^2$ or $1.020 \text{ kg}/\text{cm}^2$, as indicated in this appendix.

ABBREVIATIONS AND SYMBOLS

<i>Abbreviation</i>	<i>Full word or expression</i>
a	superscript for air
a.t.	subscript indicating active transport
Å	Ångstrom (10^{-8} cm)
A	electron acceptor
ADP	adenosine diphosphate
ATP	adenosine triphosphate
c	superscript for cuticle
cal	calorie
chl	superscript for chloroplast
clm	superscript for chloroplast limiting membranes
cm	centimeter (10^{-2} meter)
cw	superscript for cell wall
cyt	superscript for cytoplasm
C	superscript for conduction
Chl	chlorophyll
Cl	subscript for chloride ion
Cyt	cytochrome
D	electron donor
e	electron
ev	electron volt
f	farad
FAD	flavin adenine dinucleotide (oxidized form)
FADH ₂	reduced form of flavin adenine dinucleotide
g	gram
hr	hour

(continued)

<i>Abbreviation</i>	<i>Full word or expression</i>
$h\nu$	a quantum of light energy
H	subscript for heat
i	superscript for inside
ias	superscript for intercellular air spaces
in	superscript for inward
<i>in vitro</i>	in a test tube, beaker, flask (literally, <i>in glass</i>)
<i>in vivo</i>	in a living organism (literally, <i>in life</i>)
IR	infrared
j	subscript for species j
k	kilo (as a prefix), 10^3
kcal	kilocalorie
km	kilometer
K	Kelvin
	subscript for potassium ion
l	lower
ln	natural or Napierian logarithm (to the base e , where e is 2.71828...)
log	common or Briggsian logarithm (to the base 10)
m	milli (as a prefix), 10^{-3}
	meter
	superscript for cell wall pores of mesophyll cells
max	maximum
mem ^b	superscript indicating membrane
mes	superscript for mesophyll
min	minute
mm	millimeter (10^{-3} meter)
mM	millimolar
mole	a mass equal to the molecular weight of the species in grams; contains Avogadro's number of molecules
M	molar (moles/liter)
n	nano (as a prefix), 10^{-9}
nm	nanometer (10^{-9} meter)
N	normal (gram equivalent weights/liter)
Na	subscript for sodium ion
NAD	nicotinamide adenine dinucleotide (oxidized form)
NADH ₂	reduced form of nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate (oxidized form)

<i>Abbreviation</i>	<i>Full word or expression</i>
NADPH ₂	reduced form of nicotinamide adenine dinucleotide phosphate
o	superscript for outside
out	superscript for outward
p	pico (as a prefix), 10 ⁻¹²
pH	-log (<i>a</i> _{H⁺})
pl	superscript for plasmalemma
ps	superscript for photosynthesis
P	pigment
r + pr	superscript for respiration plus photorespiration
s	subscript for solute
sec	second
st	superscript for stomate(s)
sur	superscript for surroundings
S	singlet
S _(π, π)	singlet ground state
S _(π, π^*)	singlet excited state where a π electron has been promoted to a π^* orbital
ta	superscript for turbulent air
T	superscript for transpiration
	triplet
T _(π, π^*)	excited triplet state
u	upper
<i>u</i> ₊	mobility of monovalent cation
<i>u</i> ₋	mobility of monovalent anion
UV	ultraviolet
v	volt
V	subscript for volume
w	subscript for water
wv	subscript for water vapor
yr	year
δ	delta, a small quantity of something, e.g., δ^- refers to a small fraction of an electronic charge
Δ	delta, the difference or change in the quantity which follows it
$\lambda_{\max}$	wavelength position for the maximum absorption coefficient in an absorption band or for the maximum photon (or energy) emission in an emission spectrum

(continued)

<i>Abbreviation</i>	<i>Full word or expression</i>
μ	micro (as a prefix), 10^{-6}
μm	micron (10^{-6} meter)
μM	micromolar
π	Pi (ratio of circumference to diameter of a circle) refers to an electron orbital in a molecule or an electron in such an orbital
π^*	refers to an excited or antibonding electron orbital in a molecule or an electron in such an orbital
$^{\circ}$	degree Celsius (see p. 17)
	angular degree
$^{\circ}\text{K}$	degree Kelvin (absolute scale)
*	superscript referring to a standard or reference state superscript indicating a molecule in an excited electronic state
	superscript indicating saturation of air with water vapor
∞	infinity
+	subscript for cation
-	subscript for anion

NATURAL AND COMMON LOGARITHMS

The following relations and tabulated numbers are presented to facilitate calculations using both natural and common logarithms as well as their antilogarithms. For those readers who are completely unfamiliar with the properties of logarithms, a text or handbook should be consulted first.

Both the characteristics and the mantissas are given in order to speed up calculations. (Characteristics must be presented for natural logarithms.) Moreover, the minus signs in the table (for $x < 1$) refer to the entire logarithm, not just the characteristic, as they do in many common logarithm tables. Thus, $\log (0.20)$ is given as -0.699 , not as $9.301 - 10$. With appropriate interpolations, three-place accuracy can be obtained for both logarithms and antilogarithms.

$$\ln (xy) = \ln x + \ln y$$

$$\ln (x/y) = \ln x - \ln y$$

$$\ln (1/x) = -\ln x$$

$$\ln x^a = a \ln x$$

$$\ln (1/x^a) = -a \ln x$$

$$\log (xy) = \log x + \log y$$

$$\log (x/y) = \log x - \log y$$

$$\log (1/x) = -\log x$$

$$\log x^a = a \log x$$

$$\log (1/x^a) = -a \log x$$

$$\ln e = 1, \ln 10 = 2.303$$

$$\log 10 = 1, \log e = \frac{1}{2.303}$$

$$\ln e^y = y$$

$$\log 10^y = y$$

$$e^{\ln y} = y$$

$$10^{\log y} = y$$

$$\ln x = 2.303 \log x$$

$$\ln (1+x) = x - \frac{x^2}{2} + \frac{x^3}{3} - \frac{x^4}{4} + \frac{x^5}{5} - \dots \quad -1 < x \leq 1$$

$$e^x = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!} + \frac{x^4}{4!} + \dots$$

(continued)

x	$\ln x$	$\log x$	x	$\ln x$	$\log x$	x	$\ln x$	$\log x$
0.00	$-\infty$	$-\infty$	0.40	-0.916	-0.398	0.80	-0.223	-0.097
0.01	-4.605	-2.000	0.41	-0.892	-0.387	0.81	-0.211	-0.092
0.02	-3.912	-1.699	0.42	-0.868	-0.377	0.82	-0.198	-0.086
0.03	-3.507	-1.523	0.43	-0.844	-0.367	0.83	-0.186	-0.081
0.04	-3.219	-1.398	0.44	-0.821	-0.357	0.84	-0.174	-0.076
0.05	-2.996	-1.301	0.45	-0.799	-0.347	0.85	-0.163	-0.071
0.06	-2.813	-1.222	0.46	-0.777	-0.337	0.86	-0.151	-0.066
0.07	-2.659	-1.155	0.47	-0.755	-0.328	0.87	-0.139	-0.060
0.08	-2.526	-1.097	0.48	-0.734	-0.319	0.88	-0.128	-0.056
0.09	-2.408	-1.046	0.49	-0.713	-0.310	0.89	-0.117	-0.051
0.10	-2.303	-1.000	0.50	-0.693	-0.301	0.90	-0.105	-0.046
0.11	-2.207	-0.959	0.51	-0.673	-0.292	0.91	-0.094	-0.041
0.12	-2.120	-0.921	0.52	-0.654	-0.284	0.92	-0.083	-0.036
0.13	-2.040	-0.886	0.53	-0.635	-0.276	0.93	-0.073	-0.032
0.14	-1.966	-0.854	0.54	-0.616	-0.268	0.94	-0.062	-0.027
0.15	-1.897	-0.824	0.55	-0.598	-0.260	0.95	-0.051	-0.022
0.16	-1.833	-0.796	0.56	-0.580	-0.252	0.96	-0.041	-0.018
0.17	-1.772	-0.770	0.57	-0.562	-0.244	0.97	-0.030	-0.013
0.18	-1.715	-0.745	0.58	-0.545	-0.237	0.98	-0.020	-0.009
0.19	-1.661	-0.721	0.59	-0.528	-0.231	0.99	-0.010	-0.004
0.20	-1.609	-0.699	0.60	-0.511	-0.222	1.00	0.000	0.000
0.21	-1.561	-0.678	0.61	-0.494	-0.215	1.1	0.095	0.041
0.22	-1.514	-0.658	0.62	-0.478	-0.208	1.2	0.182	0.079
0.23	-1.470	-0.638	0.63	-0.462	-0.201	1.3	0.262	0.114
0.24	-1.427	-0.620	0.64	-0.446	-0.194	1.4	0.337	0.146
0.25	-1.386	-0.602	0.65	-0.431	-0.187	1.5	0.405	0.176
0.26	-1.347	-0.585	0.66	-0.416	-0.180	1.6	0.470	0.204
0.27	-1.309	-0.569	0.67	-0.400	-0.174	1.7	0.531	0.230
0.28	-1.273	-0.553	0.68	-0.386	-0.167	1.8	0.588	0.255
0.29	-1.238	-0.538	0.69	-0.371	-0.161	1.9	0.642	0.279
0.30	-1.204	-0.523	0.70	-0.357	-0.155	2.0	0.693	0.301
0.31	-1.171	-0.509	0.71	-0.342	-0.149	2.1	0.742	0.322
0.32	-1.139	-0.495	0.72	-0.329	-0.143	2.2	0.788	0.342
0.33	-1.109	-0.481	0.73	-0.315	-0.137	2.3	0.833	0.362
0.34	-1.079	-0.469	0.74	-0.301	-0.131	2.4	0.875	0.380
0.35	-1.050	-0.456	0.75	-0.288	-0.125	2.5	0.916	0.398
0.36	-1.022	-0.444	0.76	-0.274	-0.119	2.6	0.956	0.415
0.37	-0.994	-0.432	0.77	-0.261	-0.114	2.7	0.993	0.431
0.38	-0.968	-0.420	0.78	-0.248	-0.108	2.8	1.030	0.447
0.39	-0.942	-0.409	0.79	-0.236	-0.102	2.9	1.065	0.462

x	$\ln x$	$\log x$	x	$\ln x$	$\log x$	x	$\ln x$	$\log x$
3.0	1.099	0.477	7.0	1.946	0.845	20	2.996	1.301
3.1	1.131	0.491	7.1	1.960	0.851	21	3.045	1.322
3.2	1.163	0.505	7.2	1.974	0.857	22	3.091	1.342
3.3	1.194	0.519	7.3	1.988	0.863	23	3.135	1.362
3.4	1.224	0.531	7.4	2.002	0.869	24	3.178	1.380
3.5	1.253	0.544	7.5	2.015	0.875	25	3.219	1.398
3.6	1.281	0.556	7.6	2.028	0.881	26	3.258	1.415
3.7	1.308	0.568	7.7	2.041	0.886	27	3.296	1.431
3.8	1.335	0.580	7.8	2.054	0.892	28	3.332	1.447
3.9	1.361	0.591	7.9	2.067	0.898	29	3.367	1.462
4.0	1.386	0.602	8.0	2.079	0.903	30	3.401	1.477
4.1	1.411	0.613	8.1	2.092	0.908	31	3.434	1.491
4.2	1.435	0.623	8.2	2.104	0.914	32	3.466	1.505
4.3	1.459	0.633	8.3	2.116	0.919	33	3.497	1.519
4.4	1.482	0.643	8.4	2.128	0.924	34	3.526	1.531
4.5	1.504	0.653	8.5	2.140	0.929	35	3.555	1.544
4.6	1.526	0.663	8.6	2.152	0.934	36	3.584	1.556
4.7	1.548	0.672	8.7	2.163	0.940	37	3.611	1.568
4.8	1.569	0.681	8.8	2.175	0.944	38	3.638	1.580
4.9	1.589	0.690	8.9	2.186	0.949	39	3.664	1.591
5.0	1.609	0.699	9.0	2.197	0.954	40	3.689	1.602
5.1	1.629	0.708	9.1	2.208	0.959	41	3.714	1.613
5.2	1.649	0.716	9.2	2.219	0.964	42	3.738	1.623
5.3	1.668	0.724	9.3	2.230	0.968	43	3.761	1.633
5.4	1.686	0.732	9.4	2.241	0.973	44	3.784	1.643
5.5	1.705	0.740	9.5	2.251	0.978	45	3.807	1.653
5.6	1.723	0.748	9.6	2.262	0.982	46	3.829	1.663
5.7	1.741	0.756	9.7	2.272	0.987	47	3.850	1.672
5.8	1.758	0.763	9.8	2.282	0.991	48	3.871	1.681
5.9	1.775	0.771	9.9	2.293	0.996	49	3.892	1.690
6.0	1.792	0.778	10	2.303	1.000	50	3.912	1.699
6.1	1.808	0.785	11	2.398	1.041	51	3.932	1.708
6.2	1.825	0.792	12	2.485	1.079	52	3.951	1.716
6.3	1.841	0.799	13	2.565	1.114	53	3.970	1.724
6.4	1.856	0.806	14	2.639	1.146	54	3.989	1.732
6.5	1.872	0.813	15	2.708	1.176	55	4.007	1.740
6.6	1.887	0.820	16	2.773	1.204	56	4.025	1.748
6.7	1.902	0.826	17	2.833	1.230	57	4.043	1.756
6.8	1.917	0.833	18	2.890	1.255	58	4.060	1.763
6.9	1.932	0.839	19	2.944	1.279	59	4.078	1.771

(continued)

x	ln x	log x	x	ln x	log x
60	4.094	1.778	80	4.382	1.903
61	4.111	1.785	81	4.394	1.908
62	4.127	1.792	82	4.407	1.914
63	4.143	1.799	83	4.419	1.919
64	4.159	1.806	84	4.431	1.924
65	4.174	1.813	85	4.443	1.929
66	4.190	1.820	86	4.454	1.934
67	4.204	1.826	87	4.466	1.940
68	4.220	1.833	88	4.477	1.944
69	4.234	1.839	89	4.489	1.949
70	4.249	1.845	90	4.500	1.954
71	4.263	1.851	91	4.511	1.959
72	4.277	1.857	92	4.522	1.964
73	4.290	1.863	93	4.532	1.968
74	4.304	1.869	94	4.543	1.973
75	4.317	1.875	95	4.554	1.978
76	4.331	1.881	96	4.564	1.982
77	4.344	1.886	97	4.574	1.987
78	4.357	1.892	98	4.585	1.991
79	4.369	1.898	99	4.595	1.996
			100	4.605	2.000

APPENDIX SIX

CALCULUS

Functions. Derivatives of functions. Partial derivatives. Total differentials and derivatives. Well-defined and continuous functions, higher order derivatives. Examples of derivatives. Integration. Definite integrals, indefinite integrals, and the integration constant. Examples of integrals. Differential equations. References.

The purpose of this appendix is not to teach calculus, but to bring together some of the more useful relations from calculus pertinent to the quantitative aspects of physical chemistry in particular and biology in general. A few important definitions are provided to help remove some of the mystery that often surrounds the use of calculus in biology as well as to provide a convenient "refresher" for those readers whose formal coursework in this area was either rather short or long ago.

Functions. A "function" is a mathematical way of expressing the relationship between a number of quantities referred to as *variables*. One of the parameters is referred to as a *dependent* variable, which depends on one or more *independent* variables. For instance, we might have $y = mx + b$, which says that the dependent variable y equals a constant m times the value of the independent variable x plus an additive constant b . In other words, y is a function of x , and the analytical expression of the functional form is $y = mx + b$. We often find it convenient to treat such functional relationships graphically. For example, we can plot x on the x -axis, or abscissa, and y on the y -axis, or ordinate. We then obtain a straight line of slope m —an aspect to which we will return later—and with an intercept on the y -axis of b , i.e., when x is zero (as it is for the y -axis), y then equals $m \times 0 + b$, which is simply b .

The chemical potential of species j , μ_j , is a dependent variable used

throughout this text and depends on the independent variables referring to position, e.g., x for the one-dimensional case considered here, and time (t):

$$\mu_j = \mu_j(x, t) \quad (\text{VI.1})$$

where the notation $\mu_j(x, t)$ means that this function depends on x and t as independent variables. In many cases we find it convenient to express the functional relationship of the chemical potential in an analytical form with independent variables other than x and t . In particular, from Equation 2.4 we see that we can also represent the function μ_j as follows:

$$\mu_j = \mu_j(n_i, a_j, T, P, E, h) = \mu_j^* + RT \ln a_j + \bar{V}_j P + z_j F E + m_j g h \quad (\text{VI.2})$$

where n_i is necessary since the "constant" μ_j^* actually depends on the number of moles of any species present other than species j . On the other hand, R , $\bar{V}_j$, z_j , F , m_j , and g here are assumed to be constants, and do not therefore appear in the functional form for μ_j given in Equation VI.2, $\mu_j(n_i, a_j, T, P, E, h)$.

Each of the independent variables in Equation VI.2 in turn depends on position and time. For instance, if we knew the value of a_j at every location in our system for all the times of interest, we could in principle determine $a_j(x, t)$, where a_j is now a dependent variable which can be expressed in terms of the independent variables x and t . Likewise, we could find the temperature at every position and time and thereby determine $T(x, t)$. By suitably expressing the position (x) and time (t) dependence of all the independent variables in Equation VI.2 (viz., n_i , a_j , T , P , E , and h), we could convert this equation into the form given by Equation VI.1, $\mu_j = \mu_j(x, t)$. Thus, we can express the chemical potential either in terms of space and time as independent variables, or in terms of another set of physical parameters. In the latter case we obtain a useful, if approximate, analytical form for the chemical potential, while we find that the functional form $\mu_j(x, t)$ is of great utility in representing the forces on various species and the resultant fluxes.

Derivatives of functions. We are often interested in how much a function changes in value when one of the independent variables changes by a certain amount. For example, when y equals $mx + b$, by how much does y change when x increases by 1 unit? The change in y , which we will represent by Δy , where delta is used to indicate a finite change, is equal here to $m \times 1$ or m . In the more general case, when x changes by Δx , y changes by $m\Delta x$. We might also want to know the change in chemical potential of species j , μ_j , when we move across a membrane of thickness Δx , i.e., what is $\Delta\mu_j$

for a change along the x -axis of Δx ? On the other hand, we might wish to know not the gross change in μ_j across the membrane, but how the chemical potential varies over an extremely short, in fact, infinitesimally short distance. In calculus this short distance is represented by dx (the differential of x), and the dependent variable μ_j changes by $d\mu_j$ in the infinitesimal distance dx .

Next, let us consider the relationship of deltas and differentials to the slopes of lines on a graph. Let us begin by considering the functional form $y = mx + b$. What is the rate of change of y for a finite change in x ? This rate, represented by $\Delta y / \Delta x$, is $m \Delta x / \Delta x$, or simply m in this case. We have already indicated that m is the slope of the straight line represented by $y = mx + b$, so we should suspect that rates of change and slopes of curves are related. We also recognize that the total change in chemical potential across a membrane ($\Delta\mu_j$) divided by the distance over which this change occurs (Δx) gives us the average rate of change of chemical potential in the x -direction in the membrane, $\Delta\mu_j / \Delta x$. This average rate of change is illustrated by a dashed line in Figure VI.1. The dashed line has a slope $\Delta\mu_j / \Delta x$, since the dependent variable changes by $\Delta\mu_j$ when the independent variable changes by Δx . We also see that the actual values of μ_j in moving

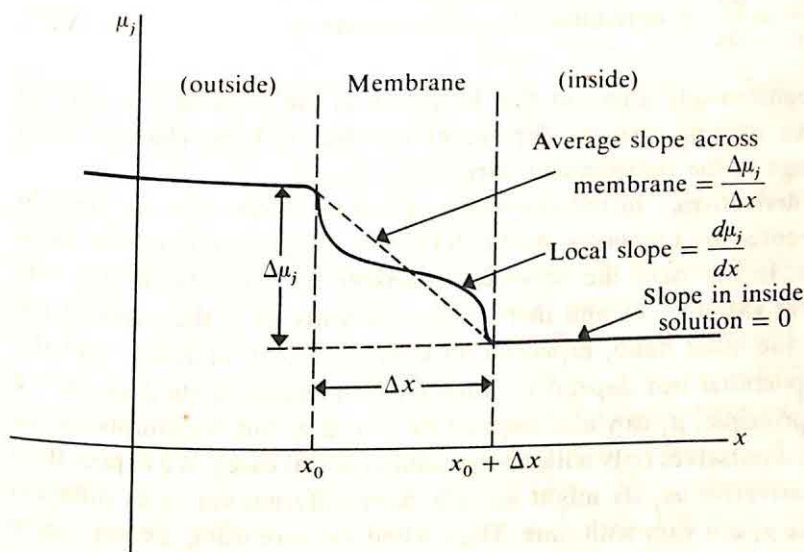


Figure VI.1

Dependence of the chemical potential of species j on position. The value of μ_j is indicated in two solutions and in a membrane (Δx thick) intervening between them.

across the membrane do not fall on the dashed line of slope $\Delta\mu_j/\Delta x$ in Figure VI.1, rather we notice that μ_j varies in a more complicated way with a change in the x -direction. This then leads us to a consideration of how μ_j varies over distances much shorter than Δx .

What is the rate of change of μ_j with position over an infinitesimally short distance dx ? This is simply $d\mu_j/dx$, which is called the *derivative* of μ_j with respect to x . When we divide one infinitesimal or differential, e.g., $d\mu_j$, by another, e.g., dx , the quotient is not an infinitesimal, rather it is the *slope of the local tangent* to the curve representing μ_j versus x at the particular location to which the $d\mu_j$ refers. For instance, to the right of the membrane in Figure VI.1 μ_j is constant, so that $d\mu_j$ is zero for any dx in this region. Hence, the slope of the line ($d\mu_j/dx$) is $0/dx$, which is zero. Within the membrane, on the other hand, the slope of μ_j as a function of x is sometimes steeper and sometimes shallower than the average rate of change of μ_j with position, $\Delta\mu_j/\Delta x$.

If we choose successively smaller values for Δx , we obtain correspondingly smaller values for the change in the dependent variable, $\Delta\mu_j$. In fact, we approach a limiting value for the ratio, $\Delta\mu_j/\Delta x$, as the value of Δx becomes vanishingly small. This then leads us to the fundamental definition of a derivative:

$$\lim_{\Delta x \rightarrow 0} \frac{\Delta\mu_j}{\Delta x} = \frac{d\mu_j}{dx} = \text{derivative of } \mu_j \text{ with respect to } x \quad (\text{VI.3})$$

We can geometrically interpret the derivative as the slope of the tangent to the curve relating how the dependent variable (μ_j here) changes for a given change in the independent variable (x here).

Partial derivatives. In the example referred to above, viz., the straight line represented by $y = mx + b$, the derivative dy/dx equals m , the slope of the line. In this case, the dependent variable (y) depends on only one independent variable (x), and there is no ambiguity as to the value of the slope. On the other hand, Equation VI.1, $\mu_j = \mu_j(x, t)$, indicates that the chemical potential can depend on time (t) in addition to its dependence on x (in principle, μ_j can also depend on y and z , but for simplicity we will concern ourselves only with the one-dimensional case). We expect then that the derivative $d\mu_j/dx$ might actually have different values at different times, since μ_j can vary with time. Thus, when we were using the derivative of μ_j only on position x was shown), we must have been implicitly referring to a specific value for t or the special case where μ_j does not depend on time.

Whenever a dependent variable depends on more than one independent variable, there are a number of different ways in which a derivative can be defined. It is convenient, however, to determine the change in the dependent variable when only one of the independent variables is allowed to vary, which leads us to a quantity known as the *partial derivative*. This is a derivative in the sense that it equals the infinitesimal change in the dependent variable divided by the infinitesimal change in some independent variable which brought about the change. It is not a total derivative, however, only a partial one, since only one of the independent variables is allowed to change. Thus, a partial derivative indicates some function's rate of change due to a change in one particular variable, all other independent variables being held constant.

Although we have indicated that the slope of μ_j versus x in Figure VI.1 is given by the derivative $d\mu_j/dx$, the slope should really be represented by the partial derivative of μ_j with respect to x , $\partial\mu_j/\partial x$, at some fixed value of time. To indicate which independent variables are held constant when we obtain a partial derivative, the symbols for a derivative are usually modified to the following form:

$$\left(\frac{\partial\mu_j}{\partial x}\right)_t = \text{partial derivative of } \mu_j \text{ with respect to } x \text{ with } t \text{ held constant} \quad (\text{VI.4})$$

We often find it convenient to specify the actual value of t at which the differentiation is made, e.g., $(\partial\mu_j/\partial x)_{t_0}$ indicates that the partial derivative is for a specific time, t_0 .

The force causing species j to move in the x -direction is represented by the negative gradient of the chemical potential, $-\partial\mu_j/\partial x$ (see p. 102). The force is positive in the direction of decreasing μ_j , so that the force in the example in Figure VI.1 is causing species j to move from the outside across the membrane to the inside. In Figure VI.1 we note that the value of $\partial\mu_j/\partial x$ changes as we cross the membrane. In particular, $-\partial\mu_j/\partial x$ is large on each side just within the membrane, indicating that the force on species j is great there. In the center of the membrane, however, the slope of the curve and consequently $-\partial\mu_j/\partial x$ are considerably less. Thus, the actual force, $-\partial\mu_j/\partial x$, differs from the average force, $-\Delta\mu_j/\Delta x$, although the latter is essentially all that we can determine in the case of biological membranes. On the other hand, the theoretical development of the relationship between forces and fluxes (pp. 100–105) requires that we deal with the actual forces as represented by the partial derivatives of the chemical potentials of the

species involved. This is one example among many where we represent forces by gradients in the appropriate potentials, a gradient being simply a partial derivative of some function with respect to position.

Partial derivatives are critically important for an adequate treatment of physical chemistry, particularly in the field of thermodynamics. For example, in Appendix VII we must evaluate the partial derivative of the chemical potential with respect to pressure, $(\partial\mu_j/\partial P)_{T,E,h,n_i,n_j}$, which we find equals $\bar{V}_j$ (Eq. VII.13). We then use this partial derivative to obtain the pressure dependence of the chemical potential in the liquid phase (Eq. VII.14), and the quite different dependence of μ_j on P that applies to a gas phase (Eq. VII.16).

Total differentials and derivatives. Initially, we presented the slope of μ_j versus x as $d\mu_j/dx$ and then refined the derivative to $(\partial\mu_j/\partial x)_t$. We will now return to the idea of an unrestricted, or total derivative; for convenience, however, we will first consider a total differential. Is it possible to represent $d\mu_j$ without any restrictions as to which variables must be held constant? The answer to this question is yes, and $d\mu_j$ assumes the following form based on Equation VI.1, $\mu_j = \mu_j(x,t)$:

$$d\mu_j = \left(\frac{\partial\mu_j}{\partial x}\right)_t dx + \left(\frac{\partial\mu_j}{\partial t}\right)_x dt \quad (\text{VI.5})$$

where $d\mu_j$ is referred to as the total differential of the function μ_j , and x and t are the independent variables on which the function depends. In other words, in order to obtain the *total differential* of some quantity (the dependent variable) we must add up contributions from each of the ways in which that quantity might change (all the independent variables). The form for the total differential is the partial derivative of the function with respect to some independent variable times the differential of that variable summed over all independent variables, as illustrated in Equation VI.5.

Having established the notion of a total differential, we will next consider a couple of examples of total derivatives. If, in a particular case, μ_j does not depend on t , $(\partial\mu_j/\partial t)_x$ equals zero. Equation VI.5 then becomes $d\mu_j = [(\partial\mu_j/\partial x)_t] dx$. Upon dividing both sides of this relation by dx , we obtain $d\mu_j/dx = (\partial\mu_j/\partial x)_t$. In fact, the total derivative equals the partial derivative only when the function whose derivative is being taken depends on but one independent variable. For instance, the slope of the curve in Figure VI.1 would indeed be $d\mu_j/dx$, if μ_j depended only on x .

Let us now examine the total derivative, $d\mu_j/dt$, when μ_j depends on both x and t . If we divide both sides of Equation VI.5 by dt , we obtain $d\mu_j/dt = (\partial\mu_j/\partial x)_t (dx/dt) + (\partial\mu_j/\partial t)_x$. The time rate of change of chemical potential

is thus the sum of the partial derivative of μ_j with respect to t at constant x , $(\partial\mu_j/\partial t)_x$, plus a contribution depending on how much our position changes in time dt and how such a change affects the chemical potential. Thus, the total derivative, $d\mu_j/dt$, is the sum of the intrinsic dependence of μ_j on t —the partial derivative, $(\partial\mu_j/\partial t)_x$ —plus our velocity of movement (dx/dt) times how rapidly μ_j changes with position $[(\partial\mu_j/\partial x)_t]$.

Well-defined and continuous functions, higher order derivatives. In order to obtain the derivatives indicated in Equations VI.3, VI.4, and VI.5, certain basic criteria must be met by the functions with which we are dealing. In the first place, μ_j must have a unique value at the particular point of interest, i.e., the chemical potential cannot have a number of different magnitudes at a given x and t , but must be a *well-defined* function. Also, if we move an infinitesimal distance along the x -axis, μ_j must change by an infinitesimal, not finite amount, a property referred to as *continuity*. If, for instance, we advance a distance dx (at a fixed time t), μ_j must change by an infinitesimal amount, $d\mu_j$. On the other hand, if μ_j changed by a finite amount, say $\Delta\mu_j$, when we moved a distance dx , then the rate of change of the chemical potential with position would be $\Delta\mu_j/dx$, which is infinite (a finite quantity divided by an infinitesimal one). This would in turn imply that we had an infinite force—the force on species j is simply $-\partial\mu_j/\partial x$ —which is certainly not the case for a situation of biological interest. We have assumed in this text that we are dealing with well-defined and continuous functions.

Often, not only must μ_j itself be well defined and continuous, but also its partial derivatives. In other words, both $(\partial\mu_j/\partial x)_t$ and $(\partial\mu_j/\partial t)_x$ must be single-valued functions and must change by an infinitesimal amount when x changes by dx , or t changes by dt . The quantity $(\partial\mu_j/\partial x)_t$ is referred to as a *first-order* partial derivative. If such a derivative is well defined and continuous, we can take its partial derivative. For instance, $(\partial\mu_j/\partial x)_t$ has two partial derivatives, $[\partial(\partial\mu_j/\partial x)_t/\partial x]_t$ and $[\partial(\partial\mu_j/\partial x)_t/\partial t]_x$, which are known as *second-order* partial derivatives. We generally represent the first of these by $(\partial^2\mu_j/\partial x^2)_t$, which is called a *homogeneous* second-order partial derivative, while the other is referred to as a *mixed* second-order partial derivative, since the derivative is taken with respect both to x and to t . When we have well-defined and continuous first-order partial derivatives, we can switch the order of differentiation in a mixed second-order partial derivative. For instance, $[\partial(\partial\mu_j/\partial x)_t/\partial t]_x$ is then equal to $[\partial(\partial\mu_j/\partial t)_x/\partial x]_t$. Such a switch in the order of differentiation is crucial for indicating how the chemical potential depends on pressure (cf. Eqs. VII.11 and VII.13).

Since the concept of higher-order derivatives has been introduced, we will briefly comment on the maxima and minima of functions. Let us suppose

that a function increases up to a maximum value and then decreases again as we move in the positive direction along the x -axis (cf. the dependence of concentration on position in Fig. 1.6). When the function reaches a maximum value, the tangent to the curve is then parallel to the x -axis and the slope of the tangent is therefore zero. We thus conclude that the first-order derivative, which is equal to the slope, is zero whenever a function attains a local maximum, e.g., at the origin in Figure 1.6. Analogously, the slope and the first-order derivative are also both zero at the point where a function has a local minimum. This useful correlation between *extrema* (maxima or minima) and the first-order derivative of a function finds many uses in physical chemistry. For example, the statement that entropy is a maximum at equilibrium means that we can describe an equilibrium situation as one where the derivative of entropy is zero (see App. VII). Another criterion for equilibrium (at constant T and P) is that it occurs for a minimum in the Gibbs free energy (cf. p. 261 and App. VII), where again the derivative—in this case, of G —is zero, e.g., $dG/dt = 0$ at equilibrium.

Whether an extremum represents a minimum or maximum can be determined from the second-order derivative. A homogeneous second-order partial derivative has a positive value at a local minimum and a negative value at a local maximum, e.g., $\partial^2 c / \partial x^2 < 0$ at the local maximum at the origin in Figure 1.6. Extrema are therefore conveniently described using the values for first-order and second-order partial derivatives.

Examples of derivatives. The following equations show various derivatives used in biological applications of calculus. In a sense, the equations are simply rules which must be followed in order to differentiate the functions indicated. Each relation can be rigorously proved. For example, we have already indicated that the derivative of a constant is zero (p. 438), which we will reconsider here for the case where the dependent variable y equals a constant b ($y = b$, which represents the equation of a straight line parallel to the x -axis at a distance b). When we move a distance dx in the positive x -direction along this line, there is no change in the value of y ($dy = 0$), hence dy/dx , which is the same as db/dx in this case, is zero. This fundamental property, that the derivative of a constant is zero, will be used when we discuss indefinite integrals and constants of integration in the next section. In the following equations a and n are constants, x , x_j , y , and t are independent variables, and u , v , w , and f are functions which can depend on x as well as on other independent variables.

$$\frac{\partial(a)}{\partial x} = \frac{d(a)}{dx} = 0$$

$$\frac{\partial(x^n)}{\partial x} = \frac{d(x^n)}{dx} = nx^{n-1}$$

$$\text{e.g., } \frac{\partial(x^1)}{\partial x} = 1x^0 = 1$$

$$\frac{\partial(x^{-1/2})}{\partial x} = -\frac{1}{2x^{3/2}}$$

$$\frac{\partial \ln x}{\partial x} = \frac{d \ln x}{dx} = \frac{1}{x}, \quad \frac{\partial \log x}{\partial x} = \frac{d \log x}{dx} = \frac{1}{2.303} \frac{d \ln x}{dx} = \frac{1}{2.303 x}$$

$$\frac{\partial(e^x)}{\partial x} = \frac{d(e^x)}{dx} = e^x$$

$$\frac{\partial u}{\partial x} = 1 / \frac{\partial x}{\partial u}$$

$$\frac{\partial(au)}{\partial x} = a \frac{\partial u}{\partial x}$$

$$\frac{\partial(u^n)}{\partial x} = nu^{n-1} \frac{\partial u}{\partial x}$$

$$\frac{\partial \ln u}{\partial x} = \frac{1}{u} \frac{\partial u}{\partial x}, \quad \frac{\partial \log u}{\partial x} = \frac{1}{2.303 u} \frac{\partial u}{\partial x}$$

$$\text{e.g., } \frac{\partial \ln ax}{\partial x} = \frac{1}{ax} \frac{\partial(ax)}{\partial x} = \frac{1}{x} \frac{\partial x}{\partial x} = \frac{1}{x}$$

$$\frac{\partial(e^u)}{\partial x} = e^u \frac{\partial u}{\partial x}$$

$$\text{e.g., } \frac{\partial(e^{ax^n})}{\partial x} = e^{ax^n} \frac{\partial(ax^n)}{\partial x} = ae^{ax^n} \frac{\partial(x^n)}{\partial x} = anx^{n-1} e^{ax^n}$$

$$\frac{\partial(u \pm v)}{\partial x} = \frac{\partial u}{\partial x} \pm \frac{\partial v}{\partial x}$$

$$\text{e.g., } \frac{\partial(\ln a + \ln x)}{\partial x} = \frac{\partial \ln a}{\partial x} + \frac{\partial \ln x}{\partial x} = 0 + \frac{1}{x} = \frac{1}{x}$$

(note that $\ln a + \ln x = \ln ax$)

$$\frac{\partial(uv)}{\partial x} = u \frac{\partial v}{\partial x} + v \frac{\partial u}{\partial x}$$

$$\text{e.g., } \frac{\partial(x^3 e^{ax})}{\partial x} = x^3 \frac{\partial(e^{ax})}{\partial x} + e^{ax} \frac{\partial(x^3)}{\partial x} = ax^3 e^{ax} + 3x^2 e^{ax}$$

$$\frac{\partial(uvw)}{\partial x} = uv \frac{\partial w}{\partial x} + uw \frac{\partial v}{\partial x} + vw \frac{\partial u}{\partial x}$$

$$\frac{\partial(u/v)}{\partial x} = \frac{1}{v} \frac{\partial u}{\partial x} - \frac{u}{v^2} \frac{\partial v}{\partial x} = \frac{v \frac{\partial u}{\partial x} - u \frac{\partial v}{\partial x}}{v^2}$$

$$\frac{\partial f(u)}{\partial x} = \frac{\partial f(u)}{\partial u} \frac{\partial u}{\partial x}$$

$$\frac{\partial}{\partial x} \left(\frac{\partial u}{\partial x} \right) = \frac{\partial^2 u}{\partial x^2}$$

$$\frac{\partial^2 f(u)}{\partial x^2} = \frac{\partial f(u)}{\partial u} \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 f(u)}{\partial u^2} \left(\frac{\partial u}{\partial x} \right)^2$$

$$\frac{\partial(a^u)}{\partial x} = a^u \ln a \frac{\partial u}{\partial x}$$

$$\frac{\partial}{\partial x} \left(\frac{\partial u}{\partial t} \right) = \frac{\partial}{\partial t} \left(\frac{\partial u}{\partial x} \right)$$

$$\begin{aligned} \text{e.g., } \frac{\partial}{\partial x} \left(\frac{\partial(x \ln at)}{\partial t} \right) &= \frac{\partial}{\partial x} \left(x \frac{\partial \ln at}{\partial t} \right) \\ &= \frac{\partial}{\partial x} \left(\frac{x}{t} \right) = \frac{1}{t} = \frac{\partial}{\partial t} \left(\frac{\partial(x \ln at)}{\partial x} \right) \\ &= \frac{\partial}{\partial t} \left(\ln at \frac{\partial x}{\partial x} \right) = \frac{\partial}{\partial t} (\ln at) = \frac{1}{t} \end{aligned}$$

$$du = \sum_j \frac{\partial u}{\partial x_j} dx_j$$

$$\text{e.g., } d(x^2 y^3) = 2xy^3 dx + 3x^2 y^2 dy$$

Integration. In addition to derivatives, in this text we also make use of a number of rather common integrals, which we will briefly consider. In a sense, integration—symbolized by $\int$, a distorted S standing for summation—is the opposite of differentiation. For example, we can differentiate μ_j to obtain the total differential, $d\mu_j$. If we now *integrate* this quantity, we obtain $\int d\mu_j$, which is μ_j , our original function. In integration, however, we must reckon with another aspect, the integration constant. To be specific, let us return to Figure VI.1, which will also help show that integration is in a sense a sophisticated adding procedure.

The chemical potential of species j is μ_j° in the solution just outside the membrane, i.e., at x_0 in Figure VI.1. As we move an infinitesimal distance dx from x_0 in the positive x direction, μ_j changes by $d\mu_j$, i.e., at $x_0 + dx$, μ_j has a value of $\mu_j^\circ + d\mu_j$ ($d\mu_j$ is negative in the present case, since μ_j

decreases as we move across the membrane in Fig. VI.1). As we go another infinitesimal distance in the positive x -direction, e.g., dx' , μ_j changes by an additional infinitesimal amount, e.g., $d\mu_j'$. Thus, in going from x_0 to $x_0 + dx + dx'$, μ_j becomes $\mu_j^o + d\mu_j + d\mu_j'$. The process of summing up these and subsequent changes in μ_j is known as integration. We are dividing up the pathway into (infinitesimally) small pieces along the x -axis and adding up all (an infinite number) of the (infinitesimal) changes occurring in μ_j in order to obtain the overall change in the chemical potential. When we get across the membrane, we expect to achieve the value that μ_j has in the inside solution, μ_j^i . We can express this process of summation, or integration, as follows:

$$\begin{aligned} \int_{x_0}^{x_0+\Delta x} d\mu_j &= \mu_j^i - \mu_j^o = \Delta\mu_j \\ &= \int_{x_0}^{x_0+\Delta x} \left(\frac{\partial\mu_j}{\partial x} \right)_t dx \end{aligned} \quad (\text{VI.6})$$

where the lower limit on the integral, x_0 , means that we begin the integration at the position where x equals x_0 , and the upper limit, $x_0 + \Delta x$, means that we terminate the integration after we cross the membrane, where our x -position is $x_0 + \Delta x$ (cf. Fig. VI.1).

We have performed the integration indicated in the upper line of Equation VI.6 by considering limits imposed on the x -position, and it might seem reasonable to represent the *integrand* $d\mu_j$ for such an integration as a function of x . To do this, we will use Equation VI.5 for the special case where μ_j does not depend on t , viz., $d\mu_j = (\partial\mu_j/\partial x)_t dx$. Thus, we can replace the integral in the upper line by $\int_{x_0}^{x_0+\Delta x} (\partial\mu_j/\partial x)_t dx$ where $(\partial\mu_j/\partial x)_t$ is the slope of the curve representing μ_j versus x (illustrated in Fig. VI.1). If we add up the products of the local values of $(\partial\mu_j/\partial x)_t$ (which gives the rate of change of chemical potential with position) times the (infinite) number of infinitesimal distances dx over which these various changes occur, then we also obtain the overall change in the function, $\Delta\mu_j$ (cf. the bottom line of Eq. VI.6).

Instead of integrating to obtain a value for $\Delta\mu_j$, we could use an addition procedure. For example, we could subdivide the section between x_0 and $x_0 + \Delta x$ into a finite number (n) of pieces each extending a distance $\Delta x/n$ along the x -axis. The local slope of the curve could be approximated by $\Delta\mu_j^k/\Delta x^k$ for the k th such subdivision, where Δx^k equals $\Delta x/n$ and $\Delta\mu_j^k$ is the change in chemical potential of species j across the k th section. In

order to obtain a value for $\Delta\mu_j$, we add up the products of such an average slope, $\Delta\mu_j^k/\Delta x^k$, times the finite width of each subdivision, $\Delta x/n$, for all n sections between x_0 and $x_0 + \Delta x$, which leads to $\sum_{k=1}^n (\Delta\mu_j^k/\Delta x^k)(\Delta x/n)$ (cf. the integral in the bottom line of Eq. VI.6). Since Δx^k equals $\Delta x/n$, the summation becomes $\sum_{k=1}^n \Delta\mu_j^k$, which is $\Delta\mu_j$, the overall change in chemical potential (cf. Eq. VI.6). This addition process—which can become quite laborious for a rapidly changing function, since the number of subdivisions (n) must then be quite large in order to match the curve closely—is known as numerical integration. The alternate procedure of integration using integrals is simply an elegant way of summing up an infinite number of infinitesimal changes in some integrand along a particular pathway of interest.

Definite integrals, indefinite integrals, and the integration constant. When an integral has both upper and lower limits as in Equation VI.6, it is referred to as a *definite* integral. When such limits are not given we have an *indefinite* integral, which can only be evaluated to within an additive constant. For example, the indefinite integral $\int d\mu_j$ equals $\mu_j + C$, where C is known as the *constant of integration*. If we impose limits on the ends of the integration pathway and hence convert the indefinite integral into a definite one, the integral would have a definite value and we would not have an additive constant of integration.

To help understand how the integration constant arises, let us return to a consideration of the integrand. We will let $d[f(x)]$ be the integrand which we wish to integrate between the limits of $x = a$ and $x = b$. But the integral of the derivative of a function is simply that function, hence we obtain the following relations:

$$\int_a^b d[f(x)] = f(x) \Big|_a^b = f(b) - f(a) \quad (\text{VI.7})$$

where the expression $f(x) \Big|_a^b$ means that we must evaluate the function at the upper limit b , i.e., $f(b)$, and then subtract its value at the lower limit, viz., $f(a)$. (We have implicitly performed this operation in Eq. VI.6.) Thus, $\int_a^b d[f(x)]$ has a *definite* value which depends on the x -positions (a and b) taken for the limits of integration.

We might wish to consider the *indefinite* integral, $\int d[f(x)]$ which does

not have defined upper and lower limits. We recognize that $f(x)$ is a "solution" to $\int d[f(x)]$, but so is $f(x) + C$, where C is an arbitrary constant. To see why this is so, let us use the rules for differentiation on p. 442: $d[f(x) + C]$ equals $d[f(x)] + dC$, which is $d[f(x)]$, since the derivative of a constant is zero ($dC = 0$). Therefore, $\int d[f(x)]$ apparently has the same value as $\int d[f(x) + C]$, which means we know the value of an indefinite integral only to within an additive constant, the so-called constant of integration. When we know the specific pathway for integration, i.e., when we have a definite integral, the constant of integration drops out, as occurs in Equations VI.6 and VI.7. As a final example, let us consider an integration pathway beginning at $x = a$ (the lower limit), going to some unspecified (variable) value of x , i.e., a definite integral with a variable upper limit. We then have $\int_a^x d[f(x)]$, which is $f(x) \Big|_a^x$, or $f(x) - f(a)$. Such an integral adds up all the changes which occur in the function $f(x)$ beginning at a specific position ($x = a$) and going to some arbitrary value of x .

Examples of integrals. Equation VI.7 not only suggests the relationship between differentiation and integration, it also embodies the basic method for evaluating integrals, viz., to find a function whose derivative is equal to the integrand. A couple of examples should help clarify this important point. For instance, what is $\int 3x^2 dx$? We know—or can determine from p. 443 with a little practice—that $d(x^3)$ equals $3x^2 dx$. Consequently, $\int 3x^2 dx$ equals $\int d(x^3)$, which is $x^3 + C$ where C is the constant of integration.

In Chapter 1 (Eq. 1.11) we had the following integral to evaluate: $\int_{c^i(0)}^{c^i(t)} dc^i / (c^0 - c^i)$. Since c^0 is a constant, $d(c^0 - c^i)$ is equal to $-dc^i$, or dc^i is the same as $-d(c^0 - c^i)$. Thus, we can re-express the integral as $\int_{c^i(0)}^{c^i(t)} -d(c^0 - c^i) / (c^0 - c^i)$. From the examples of derivatives given on page 443, we note that $d(\ln x)$ is equal to dx/x . Hence, the integral becomes $\int_{c^i(0)}^{c^i(t)} -d[\ln(c^0 - c^i)]$, which is $-\ln(c^0 - c^i) \Big|_{c^i(0)}^{c^i(t)}$, or $-\ln[c^0 - c^i(t)] + \ln[c^0 - c^i(0)]$, which in turn equals $\ln[c^0 - c^i(0)] / [c^0 - c^i(t)]$, as we indicated on p. 31.

In the following examples of integrals, a is a constant, x is an independent variable, and u and v are functions which can depend on x as well as on

other independent variables. For simplicity, the additive integration constant has been omitted from the indefinite integrals presented.

$$\int d[f(x)] = f(x)$$

$$\int a dx = a \int dx = ax$$

$$\int x^n dx = \frac{x^{n+1}}{n+1} \quad n \neq -1$$

$$\int \frac{dx}{x} = \ln x, \quad \int \frac{dx}{ax+b} = \frac{1}{a} \ln(ax+b)$$

$$\int e^x dx = e^x, \quad \int e^{ax} dx = \frac{1}{a} e^{ax}$$

$$\int x e^{ax} dx = e^{ax} \left(\frac{x}{a} - \frac{1}{a^2} \right)$$

$$\int a^x dx = \frac{a^x}{\ln a}$$

$$\int \ln x dx = x \ln x - x$$

$$\int (u \pm v) dx = \int u dx \pm \int v dx$$

$$\int u dv = uv - \int v du$$

$$\int_a^b f(x) dx = - \int_b^a f(x) dx$$

Differential equations. As a final topic in this appendix, we will consider the application of the integral calculus to the solution of differential equations. A differential equation expresses the relationship between derivatives (first-order as well as higher-order) and various variables or functions. The procedure in solving differential equations is first to put the relation into a form which can be integrated and then to carry out suitable integrations such that the derivatives are eliminated. Finally, in order to complete the solution of a differential equation, we must incorporate the known values of the functions at particular values of the variables, the so-called boundary conditions. We will illustrate the handling of differential equations by a simple, but extremely useful example.

One of the most important differential equations in biology has the following general form:

$$\frac{dy}{dt} = -ky \tag{VI.8}$$

where k is a positive constant and t represents time. We encounter this equation in Chapter 4 (Eq. 4.8) in discussing the various competing pathways for the de-excitation of an excited singlet state. Equation VI.8 also describes the process of radioactive decay, where y is the amount of radioisotope present at any time t . The equation indicates that the rate of change in time of the amount of radioactive substance (dy/dt) is linearly proportional to the amount present at that time (y). Since the radioisotope decays away in time, dy/dt is negative, hence there is a minus sign in Equation VI.8. Any process which can be described by Equation VI.8, such as a chemical reaction or even growth, is called a first-order rate process and k is known as the first-order rate constant.

To put Equation VI.8 into a form suitable for integration, we must separate the variables (y and t) so that each one appears on only one side of the equation:

$$\frac{dy}{y} = -kdt \quad (\text{VI.9})$$

which follows from Equation VI.8 upon multiplying each side by dt/y . (Note that the same initial process of separation of variables applies to the integration of a more complicated example in Ch. 3, viz., Eq. 3.11.) When the variables are separated so that a possible integrand, e.g., $-kdt$, is expressed in terms of only one variable, we can integrate that integrand. On the other hand, the integrand $-kydt$ cannot be integrated as it stands, i.e., we cannot perform $\int -kydt$, since we do not know how y depends on t . In fact, the very purpose of solving Equation VI.8 is to determine the functional relationship between y and t .

Next, we will insert integral signs into Equation VI.9 and perform the integration making use of the examples of integrals listed above (p. 448):

$$\int \frac{dy}{y} = \ln y = - \int kdt = -kt + C \quad (\text{VI.10})$$

When we take exponentials of both sides of Equation VI.10, we obtain the following expression for y :

$$e^{\ln y} = y(t) = e^{-kt+C} = e^C e^{-kt} \quad (\text{VI.11a})$$

where e^C is a constant. In Equation VI.11a we have replaced y with $y(t)$ to emphasize that y depends on the independent variable t . When t equals zero, Equation VI.11a indicates that $y(0) = e^C e^{-k \times 0}$, which is simply e^C . Thus, the constant e^C is the value taken on by the dependent variable

y when t is 0. This latter relationship, $e^C = y(0)$, is referred to as a boundary condition (or initial condition, since we are here dealing with time). Incorporating this boundary condition into Equation VI.11a, we obtain the following solution to the differential equation represented by Equation VI.8:

$$y(t) = y(0) e^{-kt} \quad (\text{VI.11b})$$

Because of the factor e^{-kt} , Equation VI.11b indicates that y decays away exponentially with time for a first-order rate process. Moreover, $y(t)$ decreases to $1/e$ of its initial value [$y(0)$] when t satisfies the following relation:

$$y(\tau) = \frac{1}{e} y(0) = y(0) e^{-k\tau} \quad (\text{VI.12})$$

where the value of time (τ) which satisfies Equation VI.12 is known as the *lifetime* of the process whose decay or disappearance is being considered. Equation VI.12 indicates that e^{-1} equals $e^{-k\tau}$, so that the first-order rate constant is equal to the reciprocal of the lifetime τ (cf. Eq. 4.11). Therefore, the solution (Eq. VI.11b) of the partial differential equation (Eq. VI.8) describing a first-order rate process becomes

$$y(t) = y(0) e^{-t/\tau} \quad (\text{VI.13})$$

References

- Crowe, A., and A. Crowe. 1969. *Mathematics for Biologists*, Academic Press, New York.
- Daniels, F. 1928 (reprinted). *Mathematical Preparation for Physical Chemistry*, McGraw-Hill, New York.
- Dwight, H. B. 1961. *Tables of Integrals and Other Mathematical Data*, 4th ed., Macmillan, New York.
- Flanders, H., R. R. Korfhage, and J. J. Price. 1970. *Calculus*, Academic Press, New York.

GIBBS FREE ENERGY AND CHEMICAL POTENTIAL

Entropy and equilibrium. Gibbs free energy. Chemical potential. Pressure dependence of μ_j . Concentration dependence of μ_j . References.

The concept of chemical potential is introduced in Chapter 2 and used throughout the rest of the book. In order not to overburden the text with mathematical details, certain points are stated without proof. Here we will justify the form of the pressure term in the chemical potential and also provide insight into how the expression for the Gibbs free energy arises.

Entropy and equilibrium. A suitable point of departure is to reconsider the condition for equilibrium. The most general statement we can make concerning the attainment of equilibrium by a system is that it occurs when the entropy of the system plus its surroundings is at a maximum. Unfortunately, entropy has proved to be a rather elusive concept to master and a difficult quantity to measure. Moreover, reference to the surroundings—the “rest of the universe” in the somewhat grandiloquent language of physics—is a nuisance. Consequently, thermodynamicists sought a function which would help describe equilibrium, but would depend only on readily measurable parameters of the system under consideration. As we shall see, the Gibbs free energy is such a function.

The concept of entropy is really part of our day-to-day observations. We know that an isolated system will spontaneously change in certain ways—a system proceeds toward a state which is more random or less ordered than the initial one. For instance, neutral solutes will diffuse toward regions where they are less concentrated. In so doing, the system lowers its capacity for further spontaneous change. For all such processes ΔS is positive, whereas ΔS becomes zero and S achieves a maximum at equilibrium. Equilibrium means that no more spontaneous changes will take place; entropy is there-

fore an *index for the capacity for spontaneous change*. It would be more convenient in some ways if entropy had been originally defined with the opposite sign. In fact, many authors introduce the quantity *negentropy*, which equals $-S$ and reaches a *minimum* at equilibrium. In any case, we must ultimately use a precise mathematical definition for entropy, such as $dS = dQ/T$, where dQ refers to the heat gain or loss in some reversible reaction taking place at temperature T .

We can represent the total entropy of the universe, S_u , as the entropy of the system under consideration, S_s , plus the entropy of the rest of the universe, S_r . We can express this in symbols as follows:

$$\begin{aligned} S_u &= S_s + S_r \\ \text{or} \\ dS_u &= dS_s + dS_r \end{aligned} \tag{VII.1}$$

An increase in S_u accompanies all real processes—this is, incidentally, the most succinct way of stating the second law of thermodynamics. S_u is maximum at equilibrium.

The heat absorbed by a system during some process is equal to the heat given up by the rest of the universe. Let us represent the infinitesimal heat exchange of the system by dQ_s . For an isothermal reaction or change, dQ_s is simply $-dQ_r$, as the heat must come from the rest of the universe. From the definition of entropy,* $dS = dQ/T$, we can obtain the following relationship:

$$dS_r = \frac{dQ_r}{T} = -\frac{dQ_s}{T} = -\frac{dU_s + PdV_s}{T} \tag{VII.2}$$

The last step in Equation VII.2 derives from the principle of the conservation of energy for the case when the only form of work involved is mechanical—a common assumption in stating the first law of thermodynamics. It is thus possible to express dQ_s as the sum of the change in internal energy (dU_s) plus a work term (PdV_s). The internal energy (U_s) is a function of the state of a system, i.e., its magnitude depends on the characteristics of the system but is independent of how the system got to that state; PV_s is also a well-defined variable. However, heat (Q_s) is not a function of the state of a system.

As we indicated above, equilibrium occurs when the entropy of the

* This definition really applies only to reversible reactions, which we can in principle use to approximate a given change—otherwise, dQ is not uniquely related to dS .

universe is maximum. This means that dS_u then equals zero (see p. 442 for a comment on derivatives and extrema of curves). By substituting Equation VII.2 into the differential form of Equation VII.1, we can express this equilibrium condition solely in terms of system parameters:

$$0 = dS_s + \left(-\frac{dU_s + PdV_s}{T} \right)$$

or

$$-TdS_s + dU_s + PdV_s = 0 \quad (\text{VII.3})$$

Equation VII.3 suggests that there is some function of the *system* which has an extremum at equilibrium. In other words, we might be able to find some expression determined by the parameters describing the system and whose derivative is zero at equilibrium. If so, the rather abstract statement that the entropy of the universe is a maximum at equilibrium could then be replaced by a "concrete" statement referring only to measurable attributes of the system—hopefully, easily measurable.

In the 1870s, J. W. Gibbs—perhaps the most brilliant thermodynamicist to date—chose a rather simple set of terms which turned out to have the very properties for which we are searching. This function is now referred to as the *Gibbs free energy*, and has the symbol G :

$$G = U + PV - TS \quad (\text{VII.4a})$$

which, upon differentiating, yields

$$dG = dU + PdV + VdP - TdS - SdT \quad (\text{VII.4b})$$

Equation VII.4b indicates that at constant temperature ($dT = 0$) and pressure ($dP = 0$), dG is

$$dG = dU + PdV - TdS \quad \text{at constant } T \text{ and } P \quad (\text{VII.5})$$

By comparing Equation VII.5 with the equilibrium condition expressed by Equation VII.3, we see that dG for a system equals zero at equilibrium at constant temperature and pressure. Moreover, G depends only on U , P , V , T , and S of the *system*. It turns out that the extremum condition, $dG = 0$, actually occurs when G reaches a minimum at equilibrium. This useful attribute of the Gibbs free energy is strictly valid only at *constant temperature and pressure*, conditions which closely approximate those encountered in many biological situations. Thus, our criterion for equilibrium shifts from a maximum of the entropy of the universe to a minimum in the Gibbs free energy of the system.

Gibbs free energy. We will now consider how the internal energy (U) changes when material enters or leaves a system. This will help us derive an expression for the Gibbs free energy which is quite useful for biological applications.

The internal energy of a system changes when substances enter or leave it. For convenience, we will consider a system of fixed volume and at the same temperature as the surroundings, so that there are no heat exchanges. If dn_j moles of species j enter such a system, U increases by $\mu_j dn_j$, where μ_j is an intensive variable representing the free energy contribution to the system per mole of species j entering or leaving. Work is often expressed as the product of an intensive quantity (such as μ_j , P , T , E , h) times an extensive one (dn_j , dV , dS , dQ , dm , respectively), i.e., the amount of any kind of work depends on both some thermodynamic parameter characterizing the internal state of the system and the extent or amount of change for the system. In our present example the extensive variable describing the amount of change is dn_j , while μ_j represents the contribution to the internal energy of the system per mole of species j . When more than one species crosses the boundary of our system, which is at constant volume and the same temperature as the surroundings, the term $\sum_j \mu_j dn_j$ is added to dU , where dn_j is positive if the species enters the system and negative if it leaves. In the general case, when we consider all the ways that the internal energy of a system can change, we can represent dU as follows:

$$dU = dQ - PdV + \sum_j \mu_j dn_j \quad (\text{VII.6})$$

Let us now return to the development of a useful relation for the Gibbs free energy of a system. When dU as expressed by Equation VII.6 is substituted into dG as given by Equation VII.5, we obtain

$$\begin{aligned} dG &= TdS - PdV + \sum_j \mu_j dn_j + PdV - TdS \\ &= \sum_j \mu_j dn_j \end{aligned} \quad (\text{VII.7})$$

where dQ has been replaced by TdS . Equation VII.7 indicates that the particular form chosen for the Gibbs free energy leads to a very simple expression for dG at constant T and P , viz., dG then depends only on μ_j and dn_j .

In order to obtain an expression for G , we must integrate Equation VII.7.

To facilitate the integration we will define a new variable, α , such that dn_j equals $n_j d\alpha$, where n_j is the total number of moles of species j present in the final system, i.e., n_j is a constant describing the final system. The subsequent integration from $\alpha = 0$ to $\alpha = 1$ corresponds to building up the system by a simultaneous addition of all the components in the same proportions present in the final system. (The intensive variable μ_j is also held constant for this integration pathway, i.e., the chemical potential of species j does not depend on the size of the system.) Using Equation VII.7 and this rather easy integration pathway, we obtain the following expression for the Gibbs free energy:

$$G = \int dG = \int \sum_j \mu_j dn_j = \int_0^1 \sum_j \mu_j n_j d\alpha = \sum_j \mu_j n_j \int_0^1 d\alpha = \sum_j \mu_j n_j \quad (\text{VII.8})$$

The well-known relation between G and the μ_j 's in Equation VII.8 can also be obtained by a rigorous method which is more elegant mathematically, but somewhat involved.

In Chapter 6 we presented without proof an expression for the Gibbs free energy (Eq. VII.8 is identical to Eq. 6.1) and also noted some of the properties of G . For instance, at constant temperature and pressure, the direction for a spontaneous change is toward a lower Gibbs free energy; minimum G is achieved at equilibrium. Hence, ΔG is negative for such spontaneous processes. Spontaneous processes can in principle be harnessed to do useful work, where the maximum amount of work possible at constant temperature and pressure is equal to the absolute value of ΔG (some of the energy is dissipated by inevitable inefficiencies such as frictional losses, so that $-\Delta G$ represents the *maximum* work possible). To drive a reaction in the direction opposite to that in which it proceeds spontaneously requires a free energy input of at least ΔG .

Chemical potential. Let us now examine the properties of the intensive variable μ_j . Equation VII.8 ($G = \sum_j \mu_j n_j$) suggests a very useful way of defining μ_j . In particular, if we keep the μ_i and n_i constant, we obtain the following expression:

$$\begin{aligned} \mu_j &= \left(\frac{\partial G}{\partial n_j} \right)_{\mu_i, n_i} \\ &= \left(\frac{\partial G}{\partial n_j} \right)_{T, P, E, h, n_i} \end{aligned} \quad (\text{VII.9})$$

where n_i and μ_i refer to all species other than species j . Since μ_i can depend on T , P , E (the electrical potential), h (the height in a gravitational field), and n_i , the act of keeping μ_i constant during partial differentiation is the same as keeping T , P , E , h , and n_i constant, as is indicated in the second line of Equation VII.9. Equation VII.9 indicates that the chemical potential of species j is the partial molal Gibbs free energy of a system with respect to that species, and that it is obtained when T , P , E , h , and the amount of all other species are held constant. Thus, μ_j corresponds to the intensive contribution of species j to the extensive quantity G , the Gibbs free energy of the system.

In Chapter 2 (pp. 58–62, 80) and Chapter 3 (p. 92) we argued that μ_j depends on T , a_j ($a_j = \gamma_j c_j$, Eq. 2.5), P , E , and h in a solution, while the partial pressure of species j , P_j , is also involved for the chemical potential in a vapor phase. We can summarize the two relations as follows:

$$\begin{aligned}\mu_j^{\text{liquid}} &= \mu_j^* + RT \ln a_j + \bar{V}_j P + z_j FE + m_j gh \\ \mu_j^{\text{vapor}} &= \mu_j^* + RT \ln \frac{P_j}{P_j^*} + m_j gh\end{aligned}\quad (\text{VII.10})$$

The forms for the gravitational contribution ($m_j gh$) and the electrical one ($z_j FE$) can easily be understood. We showed in Chapter 3 (p. 105) that $RT \ln a_j$ is the correct form for the concentration term in μ_j . The reasons for the forms of the pressure terms in a liquid ($\bar{V}_j P$) and in a gas [$RT \ln (P_j/P_j^*)$] are not so obvious. Therefore, we will examine the pressure dependence of the chemical potential of species j in some detail.

Pressure dependence of μ_j . In order to derive the pressure terms in the chemical potentials of solvents, solutes, and gases, we must rely on certain properties of partial derivatives as well as commonly observed effects of pressure. To begin with, we will differentiate the chemical potential in Equation VII.9 with respect to P :

$$\begin{aligned}\left(\frac{\partial \mu_j}{\partial P}\right)_{T,E,h,n_i,n_j} &= \left[\frac{\partial}{\partial P} \left(\frac{\partial G}{\partial n_j} \right)_{T,P,E,h,n_i} \right]_{T,E,h,n_i,n_j} \\ &= \left[\frac{\partial}{\partial n_j} \left(\frac{\partial G}{\partial P} \right)_{T,E,h,n_i,n_j} \right]_{T,P,E,h,n_i}\end{aligned}\quad (\text{VII.11})$$

where we have reversed the order for partial differentiation with respect to P and n_j —this is permissible for functions such as G , which have well-defined and continuous first-order partial derivatives, cf. p. 441. The differential form of Equation VII.4 ($dG = dU + PdV + VdP - TdS - SdT$) gives us a suitable form for dG . If we substitute dU given by Equation VII.6

($dU = TdS - PdV + \sum_j \mu_j dn_j$, where dQ is replaced by TdS , cf. p. 452) into this expression for the derivative of the Gibbs free energy, we can express dG in the following useful form:

$$dG = VdP - SdT + \sum_j \mu_j dn_j \quad (\text{VII.12})$$

Using Equation VII.12 we can readily determine the pressure dependence of the Gibbs free energy as needed in the last bracket of Equation VII.11. Namely, $(\partial G/\partial P)_{T,E,h,n_i,n_j}$ equals V by Equation VII.12. Next, we have to consider the partial derivative of this V with respect to n_j (cf. bottom line of Eq. VII.11). But Equation 2.6 indicates that $(\partial V/\partial n_j)_{T,P,E,h,n_i}$ is $\bar{V}_j$, the partial molal volume. Substituting these partial derivatives into Equation VII.11 leads us to the following useful expression:

$$\left(\frac{\partial \mu_j}{\partial P}\right)_{T,E,h,n_i,n_j} = \bar{V}_j \quad (\text{VII.13})$$

Equation VII.13 is of pivotal importance in deriving the form of the pressure term in the chemical potentials of both liquid and vapor phases.

Let us first consider an integration of Equation VII.13 appropriate for a liquid. We will make use of the observation that the partial molal volume of a species in a solution does not depend on the pressure to any significant extent. For a solvent this means that the liquid is generally rather incompressible. If we integrate Equation VII.13 at constant T, E, h, n_i and n_j with $\bar{V}_j$ independent of P , we obtain the following relations:

$$\begin{aligned} \int \frac{\partial \mu_j}{\partial P} dP &= \int_{\mu_j^*}^{\mu_j^{\text{liquid}}} d\mu_j = \mu_j^{\text{liquid}} - \mu_j^* \\ &= \int \bar{V}_j dP = \bar{V}_j \int dP = \bar{V}_j P + \text{"constant"} \end{aligned} \quad (\text{VII.14})$$

where the definite integral in the top line is taken from the chemical potential of species j in a standard state as the lower limit, up to the general μ_j in a liquid as the upper limit. The bottom line contains an indefinite integral—see p. 446 for definitions of definite and indefinite integrals. The integration of $\int \bar{V}_j dP$ leads to our pressure term $\bar{V}_j P$ plus a constant. Since the integration was performed while holding T, E, h, n_i , and n_j fixed, the “constant” can depend on all of these variables, but not on P .

Equation VII.14 indicates that the chemical potential of a liquid contains

a pressure term of the form $\bar{V}_j P$. The other terms (in particular, μ_j^* , $RT \ln a_j$, $z_j FE$, and $m_j gh$) do not depend on pressure, a consideration used throughout this text. The experimental observation which gives us this very useful form for μ_j is that $\bar{V}_j$ is generally not influenced very much by pressure, e.g., a liquid is often essentially incompressible. If this does not prove valid under certain situations, $\bar{V}_j P$ would not be a suitable term in the chemical potential of species j for expressing the pressure dependence in a solution.

Next, we turn to the form of the pressure term in the chemical potential of a gas, where the assumption of incompressibility that we used for a liquid is obviously not valid. Our point of departure is the perfect gas law:

$$P\bar{V}_j = RT \quad (\text{VII.15})$$

where $\bar{V}_j$ is the volume per mole of gas of species j . The experimental observation introduced in this case is that real gases behave to a large extent like ideal gases. When Equation VII.13 is integrated at constant T , h , n_i , and n_j , using the gas law to yield the value of $\bar{V}_j$, we find that the chemical potential of gaseous species j depends on the *logarithm* of the pressure:

$$\begin{aligned} \int \frac{\partial \mu_j}{\partial P} dP &= \int_{\mu_j^*}^{\mu_j^{\text{vapor}}} d\mu_j = \mu_j^{\text{vapor}} - \mu_j^* \\ &= \int \bar{V}_j dP = \int RT \frac{dP}{P} = RT \ln P + \text{"constant"} \end{aligned} \quad (\text{VII.16})$$

where the "constant" in the bottom line can depend on T , h , n_i , and n_j .

To put Equation VII.16 into a more useful form, we will incorporate the additivity of partial pressures. In particular, P equals $\sum_j P_j$, where P_j is the partial pressure due to species j . Since the logarithm of a product is the sum of the individual logarithms (cf. App. V), $\ln P$ then equals $\ln P_j$ plus $\sum_{i \neq j} \ln P_i$, where the sum is over all species except species j . It is useful to

modify Equation VII.16 in yet another way, i.e., to add and subtract $RT \ln P_j^*$, where P_j^* is the saturation partial pressure of species j (see p. 80). We can then rewrite Equation VII.16 as follows, summarizing our various substitutions:

$$\begin{aligned} \mu_j^{\text{vapor}} &= \mu_j^* + RT \ln P + \text{"constant"} \\ &= \mu_j^* + RT \ln P_j + RT \sum_{i \neq j} \ln P_i + \text{"constant"} \end{aligned}$$

$$\begin{aligned}
 &= \mu_j^* + RT \ln P_j - RT \ln P_j^* + RT \ln P_j^* + RT \sum_{i \neq j} \ln P_i \\
 &\quad + \text{"constant"} \\
 &= \mu_j^* + RT \ln \frac{P_j}{P_j^*} + m_j g h \quad (\text{VII.17})
 \end{aligned}$$

where $RT \ln P_j^* + RT \sum_{i \neq j} \ln P_i + \text{"constant"}$ is actually equal in this case

to $m_j g h$. Equation VII.17, which we used without proof on p. 81, shows the *logarithmic* dependence of the chemical potential of gaseous species j on its partial pressure, which is in marked contrast to the *linear* dependence on pressure for a liquid, Equation VII.14.

Concentration dependence of μ_j . We will complete our discussion of chemical potential by using Equation VII.17 to obtain the logarithmic term in concentration which is found for μ_j in a liquid phase. First, it should be pointed out that Equation VII.17 has no concentration term *per se* for the chemical potential of species j in a gas phase. However, the partial pressure of a species in a gas phase is really analogous to the concentration of a species in a liquid, e.g., $P_j V = n_j RT$ for gaseous species j , and concentration means number/volume = $n_j/V = P_j RT$.

Raoult's law states that at equilibrium the partial pressure of a particular gas above its volatile liquid is proportional to the mole fraction of that solvent in the liquid phase. A similar relation more appropriate for solutes is Henry's law, which states that P_j in the vapor phase is proportional to the N_j of that solute in the liquid phase. Although the proportionality coefficients in the two relations are different, they both indicate that P_j^{vapor} depends linearly on N_j^{solution} . For dilute solutions the concentration of species j , c_j , is proportional to its mole fraction, N_j (this is true for both solute and solvent). Thus, when P_j^{vapor} changes from one equilibrium condition to another, we expect a similar change in c_j^{solution} , since μ_j^{liquid} equals μ_j^{vapor} at equilibrium. In particular, Equation VII.17 indicates that μ_j^{vapor} depends on $RT \ln (P_j/P_j^*)$, hence the chemical potential of a solvent or solute should contain a term of the form $RT \ln c_j$, as it does in fact (cf. Eqs. 2.4 and VII.10). As we discussed on p. 60, we should be concerned about the concentration which is thermodynamically active, a_j ($a_j = \gamma_j c_j$, Eq. 2.5), so that the actual term appearing in the chemical potential for a solute or solvent is $RT \ln a_j$, not $RT \ln c_j$. In Chapter 3 (p. 105), instead of the present argument based on Raoult's and Henry's laws, we used a comparison with Fick's first law to justify the $RT \ln a_j$ term. Moreover, the Boyle-Van't Hoff relation, which was derived *assuming* the $RT \ln a_j$ term,

more plausible, we will transform the dissipation function from our previous set of forces and fluxes to a more convenient one involving J_v , J_D , ΔP , and $\Delta\pi$.

We will begin by simply stating how the dissipation function (Φ) can be expressed. This function, which is a measure of the rate at which energy is irreversibly dissipated in a system, equals the sum of the various fluxes (J_k) times their conjugate forces (X_k), i.e., $\Phi = \sum_k J_k X_k$. Let us consider the dissipation function for the conjugate forces and fluxes used in Chapter 3:

$$\Phi = J_w \Delta\mu_w + J_s \Delta\mu_s \quad (\text{VIII.1})$$

Using Equations 3.31 ($\Delta\mu_w = \bar{V}_w \Delta P - \bar{V}_w \Delta\pi$) and 3.32 ($\Delta\mu_s = \bar{V}_s \Delta P + \frac{1}{\bar{c}_s} \Delta\pi$), we can rewrite Equation VIII.1 as follows:

$$\begin{aligned} \Phi &= J_w (\bar{V}_w \Delta P - \bar{V}_w \Delta\pi) + J_s \left(\bar{V}_s \Delta P + \frac{1}{\bar{c}_s} \Delta\pi \right) \\ &= (J_w \bar{V}_w + J_s \bar{V}_s) \Delta P + \left(\frac{J_s}{\bar{c}_s} - J_w \bar{V}_w \right) \Delta\pi \\ &= J_v \Delta P + J_D \Delta\pi \end{aligned} \quad (\text{VIII.2})$$

where J_v is the total volume flow as defined in Chapter 3 (Eq. 3.33, $J_v = \bar{V}_w J_w + \bar{V}_s J_s$). Since the dissipation function is by definition simply the sum of the various fluxes times their conjugate "forces," from the last line of Equation VIII.2 we can identify J_v as being conjugate to ΔP , and J_D to $\Delta\pi$. Thus, we have already transformed Φ into our new set of conjugate forces and fluxes.

We can readily show that the newly introduced diffusional flux (J_D) is the difference between the average velocities of the solute (v_s) and water (v_w). We begin by noting that $\bar{V}_w c_w$ is essentially unity for a dilute aqueous solution ($\bar{V}_w c_w + \bar{V}_s c_s = 1$ for a solution of a single solute, where $\bar{V}_s c_s \ll 1$ for a dilute solution), hence $J_w \bar{V}_w$ is approximately equal to J_w / c_w . But the average velocity of species j , v_j , is simply J_j / c_j (see p. 103 for details). Hence, J_D as defined by Equation VIII.2 can be re-expressed as follows:

$$\begin{aligned} J_D &= J_s / \bar{c}_s - J_w \bar{V}_w \\ &\cong J_s / \bar{c}_s - J_w / c_w \\ &= v_s - v_w \end{aligned} \quad (\text{VIII.3})$$

The diffusional flux given in Equation VIII.3 is the difference between the mean velocities of solute and water. In mass flow, e.g., that described

by Poiseuille's law (Eq. 8.9), v_s equals v_w , so J_D is zero; such flow is independent of $\Delta\pi$ and depends only on the gradient in hydrostatic pressure. On the other hand, let us consider $\Delta\pi$ across a membrane which greatly restricts the passage of some solute relative to the movement of water, i.e., a barrier which acts as a differential filter; v_s would then be considerably less than v_w , so that J_D would have a non-zero value in response to its conjugate "force," $\Delta\pi$. Thus, J_D expresses the tendency of the solute relative to water to diffuse in response to a gradient in osmotic pressure.

Interdependence of forces and fluxes, reflection coefficients. Next, we will use our new set of parameters to express the linear interdependence of conjugate forces and fluxes in irreversible thermodynamics (Eq. 3.28, $J_j = \sum_k L_{jk} X_k$):

$$J_V = L_P \Delta P + L_{PD} \Delta \pi \quad (\text{VIII.4})$$

$$J_D = L_{DP} \Delta P + L_D \Delta \pi \quad (\text{VIII.5})$$

where the subscripts in both equations are those generally used in the literature. (Although these subscripts— P referring to pressure and D to diffusion—are not consistent with the L_{jk} convention, four coefficients are still needed to describe the dependence of two fluxes on their conjugate forces.) By the Onsager reciprocity relation, which is applicable in the present case of conjugate forces and fluxes, L_{PD} equals L_{DP} , and again only three different coefficients are needed to describe the movement of water and a single solute across a membrane (cf. Eqs. 3.29 and 3.30).

Equation VIII.4 is quite similar to Equation 3.38 [$J_V = L_P(\Delta P - \sigma \Delta \pi)$]. In particular, L_P is the same hydraulic conductivity coefficient in both cases. It describes the mechanical filtration capacity of the membrane or other barrier, i.e., when $\Delta\pi$ is zero, L_P relates the total volume flow (J_V) to the hydrostatic pressure difference (ΔP). When ΔP is zero, Equation VIII.5 indicates that a difference in osmotic pressure leads to a diffusional flow characterized by the coefficient L_D . Membranes also generally exhibit a property called *ultrafiltration*, whereby they offer different resistances to the passage of the solute and water. For instance, in the absence of an osmotic pressure drop ($\Delta\pi = 0$), Equation VIII.5 indicates that there will be a diffusional flux equal to $L_{DP} \Delta P$. Based on Equation VIII.3 ($J_D = v_s - v_w$), v_s is then different from v_w , which would result if the membrane restricted the passage of solute more than that of water. Thus, the phenomenological coefficient L_{DP} helps describe the relative ease with which solute crosses the membrane, compared with water. But such a property of relative selectivity by a barrier is also embodied in the reflection coefficient, σ (pp. 144–148). In fact, A. J. Staverman in 1951 defined the reflection coefficient

in terms of L_{DP} as follows:

$$\sigma = -L_{DP}/L_P = -L_{PD}/L_P \quad (\text{VIII.6})$$

Equation VIII.6 is a considerably simpler definition of the reflection coefficient than is the equivalent one given by Equation 3.37.

When a membrane is nonselective, v_s equals v_w and $\Delta\pi$ is zero (cf. p. 146). If v_s equals v_w , then J_D must be zero by Equation VIII.3. For this to be true for any ΔP and a zero $\Delta\pi$, Equation VIII.5 indicates that L_{DP} must be zero, hence the reflection coefficient is also zero in this case ($\sigma = -L_{DP}/L_P$, Eq. VIII.6). Thus, σ is zero when the membrane does not select between solute and solvent. At the opposite extreme, the solute does not cross the membrane ($v_s = 0$), hence J_D is $-v_w$ ($J_D = v_s - v_w$, Eq. VIII.3). Moreover, J_v , which is the average velocity of the solution (cf. p. 393), is then simply v_w . Thus, J_v equals $-J_D$ when the solute does not cross the membrane, and $L_P\Delta P + L_{PD}\Delta\pi$ equals $-L_{DP}\Delta P - L_D\Delta\pi$ by Equations VIII.4 and VIII.5. Since this is true for any ΔP , L_P must then equal $-L_{DP}$, hence $-L_{DP}/L_P$ equals unity in this case. The reflection coefficient is therefore unity ($\sigma = -L_{DP}/L_P$, Eq. VIII.6) when the solute does not cross the membrane. Consequently, both the limits of nonselectivity ($\sigma = 0$) and impermeability ($\sigma = 1$) can easily be presented using the forces and fluxes adopted in this appendix.

Using the definition of σ (Eq. VIII.6), we can rewrite Equation VIII.4 to obtain the following form for the total volume flow:

$$J_v = L_P(\Delta P - \sigma\Delta\pi) \quad (\text{VIII.7})$$

This equation is identical to Equation 3.38, further indicating that L_P and σ have the same meaning in the two different approaches. Equation VIII.7 can likewise be generalized to include the case of more than one solute—cf. Equation 3.39, $J_v = L_P(\Delta P - \sum_j \sigma_j \Delta\pi_j)$.

Irreversible thermodynamic description of solute flux. Our final objective is to obtain an expression for the solute flux (J_s) which takes into consideration the coupling of forces and fluxes of irreversible thermodynamics. We will begin by showing that $J_D + J_v$ is approximately equal to J_s/c_s . By Equation VIII.3, J_D equals $J_s/\bar{c}_s - J_w \bar{V}_w$, while J_v is $J_w \bar{V}_w + J_s \bar{V}_s$ (Eq. 3.33). For a dilute solution, $\bar{V}_s c_s \ll 1$ or $1/c_s \gg \bar{V}_s$, so that we obtain the following relations:

$$\begin{aligned} J_D + J_v &= J_s/\bar{c}_s - J_w \bar{V}_w + J_w \bar{V}_w + J_s \bar{V}_s \\ &= J_s \left(\frac{1}{\bar{c}_s} + \bar{V}_s \right) \\ &\cong J_s/\bar{c}_s \end{aligned} \quad (\text{VIII.8})$$

After multiplying both sides of Equation VIII.8 by $\bar{c}_s$, and using Equation VIII.5 for J_D , we obtain:

$$\begin{aligned}
 J_s &= \bar{c}_s J_v + \bar{c}_s J_D \\
 &= \bar{c}_s J_v + \bar{c}_s L_{DP} \Delta P + \bar{c}_s L_D \Delta \pi \\
 &= \bar{c}_s J_v + \bar{c}_s L_{DP} \frac{(J_v - L_{PD} \Delta \pi)}{L_P} + \bar{c}_s L_D \Delta \pi \quad (\text{VIII.9}) \\
 &= \bar{c}_s J_v \left(1 + \frac{L_{DP}}{L_P} \right) + \bar{c}_s \Delta \pi \left(L_D - \frac{L_{DP} L_{PD}}{L_P} \right)
 \end{aligned}$$

where Equation VIII.4 is used in the second equality to express ΔP . Finally, we combine the factors multiplying $\Delta \pi$ into a new coefficient for solute permeability, ω_s , and use the previous definition of σ (Eq. VIII.6), so that we can rewrite the last line of Equation VIII.9 as:

$$J_s = \bar{c}_s (1 - \sigma) J_v + \omega_s \Delta \pi \quad (\text{VIII.10})$$

Equation VIII.10 indicates that J_s not only depends on $\Delta \pi$, as would be expected on the basis of classical thermodynamics, but also that the flux of solute can be affected by the overall volume flux, J_v . In particular, the classical expression for J_s for a neutral solute is $P_j \Delta c_j$ (Eq. 1.8), which equals $(P_j/RT) \Delta \pi_j$ using the Van't Hoff relation (Eq. 2.10, $\pi_s = RT \sum_j c_j$). Thus,

ω_s is analogous to P_j/RT of the classical thermodynamic description. The classical treatment indicates that J_s is zero if $\Delta \pi$ is zero. On the other hand, when $\Delta \pi$ is zero, Equation VIII.10 indicates that J_s equals $\bar{c}_s (1 - \sigma) J_v$, therefore P_j may not always be an adequate parameter by which to describe the permeability of species j . Finally, in the case of a single solute, movement across a membrane is fully characterized by the three experimentally convenient parameters introduced by irreversible thermodynamics: L_P , σ , and ω_s .

References

- Katchalsky, A., and P. F. Curran. 1967. *Nonequilibrium Thermodynamics in Biophysics*, Harvard University Press, Cambridge.
- Kedem, O., and A. Katchalsky. 1958. Thermodynamic analysis of the permeability of biological membranes to nonelectrolytes. *Biochimica et Biophysica Acta* 27: 229-246.
- Stein, W. D. 1967. *The Movement of Molecules Across Cell Membranes*, Academic Press, New York.

MEASUREMENT OF ELECTRICAL POTENTIAL DIFFERENCES ACROSS BIOLOGICAL MEMBRANES

Making and filling of micropipettes. Electrical circuitry. Examining cells. Experimental measurements. References.

For the Nernst equation (Eq. 3.5), the diffusion potential in a simple solution (Eq. 3.10), the Goldman equation (Eq. 3.19), the Donnan potential (pp. 116–118), and the Ussing-Teorell equation (Eq. 3.24), three important experimental parameters are the external concentration of some ion, its internal concentration, and the electrical potential difference existing between two phases. Of these quantities, the external concentration is either readily measured or held constant by the experimenter. Measurement of the internal concentration is generally quite difficult. Determining electrical potential differences is intermediate in difficulty, and such determinations can be used to obtain information on certain internal concentrations.

In order to observe the electrical potential difference across a membrane, we must establish an electrical connection with both the outside and inside of a cell. To connect electrically to the inside of a cell, a very fine hollow glass tube is used, known as a micropipette (also referred to as a microsalt-bridge or microelectrode). It is filled with an electrically conducting solution and connected via a half-cell to one side of a device by which electrical potentials can be measured. In order not to disturb the cell, the tip of the micropipette must be small. This causes it to have a high electrical resistance and requires that the electrical circuitry be capable of measuring a small voltage in the presence of a large resistance.

Making and filling of micropipettes. Micropipettes can be made from pieces of capillary glass about 100 mm long with an internal diameter near

1.0 mm. The glass is heated in the center and then carefully pulled apart to form two micropipettes. The shape of the tip, which should be $1\text{ }\mu\text{m}$ or less in diameter, depends on the temperature of the heater coil, the force applied in pulling the micropipettes, and the type of glass used. After cooling, the micropipettes are carefully removed from the puller and placed in a suitable holder. The fine tips are extremely fragile and can even be broken by the impact of air-borne dust particles.

We generally fill the hollow capillaries with 3 M KCl. This solution is a good electrical conductor; also K^+ and Cl^- have similar mobilities so that the diffusion potential across the micropipette tip is minimized (cf. p. 108). Filling is conveniently achieved by submerging the micropipettes (tip up) in a preheated flask containing 3 M KCl, e.g., a wide-mouth 1000 ml pyrex Erlenmeyer flask equipped with a three-hole stopper and containing 800 ml of 3 M KCl at 90° . One hole of the stopper is for attachment to a vacuum line, one contains a short piece of glass tubing open to the air, and the third hole is for the support rod of the micropipette holder. This holder can have slots around its periphery in which the micropipettes are placed vertically and then held by a rubber band running horizontally in a groove. A slight vacuum is gradually applied so that the solution boils for a few seconds. The initial decrease in pressure causes the air in the micropipettes to expand and bubble out, and the subsequent boiling causes the space previously occupied by air to be replaced by water vapor. When we remove the vacuum the gas in the micropipettes contracts and the water vapor condenses upon cooling, leading to a filling of the micropipette. By applying and releasing the vacuum a few times, bubbles will eventually cease to emerge from the micropipettes, indicating that they are filled. (When illuminated at right angles to the line of sight, bubbles remaining in the tips appear as dark spots.) The filled micropipettes are stored in 3 M KCl.

Electrical circuitry. The base of a filled micropipette is electrically connected to a half-cell with a suitable saltbridge. This connection is conveniently made using flexible tubing with an internal diameter near 0.6 mm, filled with 2% agar in 3 M KCl. The agar prevents macroscopic flow, but not diffusional movement of the electrolyte, while the use of KCl as the conducting solution in our saltbridges minimizes unwanted diffusion potentials. The 2% agar is melted, drawn into a syringe, e.g., a $2\frac{1}{2}$ ml syringe with a 2.5 cm needle, and then injected into the tubing. To prevent solidification while filling, we can place the tubing under warm water; the emerging air bubbles can be used to assess the extent of filling. With 3 M KCl as the electrolyte, a 30 cm length of tubing should have a resistance of less than 10^5 ohms, whereas the micropipette into which it is inserted should

have a resistance of at least 10^6 ohms—the finer the tip, the higher the resistance.

To connect an electrical potential measuring device into the circuit, the electrical conductor must be changed from an electrolyte solution to a metal wire. (Charge moves as ions in a solution and as electrons in a metal.) This transition is easily accomplished using half-cells. A suitable half-cell consists of some metal, e.g., Ag or Hg, coated with an insoluble salt of that metal (AgCl or HgCl) and placed in a solution containing the anion (Cl^-) of the salt. When electrons flow along the wire into the metal, the Ag^+ or Hg^+ is reduced to Ag or Hg, respectively, and the Cl^- passes out into the solution. Since the current can be in either direction, such a half-cell is called “reversible.”

Two half-cells are needed to complete the electrical circuit. One half-cell is put into a beaker containing 3 M KCl and the tubing saltbridge (filled with 2% agar in 3 M KCl) coming from the micropipette, as illustrated in part (a) of Figure IX.1. An identical half-cell is put in a second beaker containing 3 M KCl and another filled tubing saltbridge. (The other end of this tubing is placed in the solution bathing the cell whose electrical potential differences are to be determined.) The electrical potential measuring device is now connected using the wires coming from each of the two half-cells (see Fig. IX.1a). Half-cells have a fixed and characteristic potential drop across them; when identical half-cells are placed as in Figure IX.1, these ΔE s occur in opposite directions and therefore tend to cancel each other.

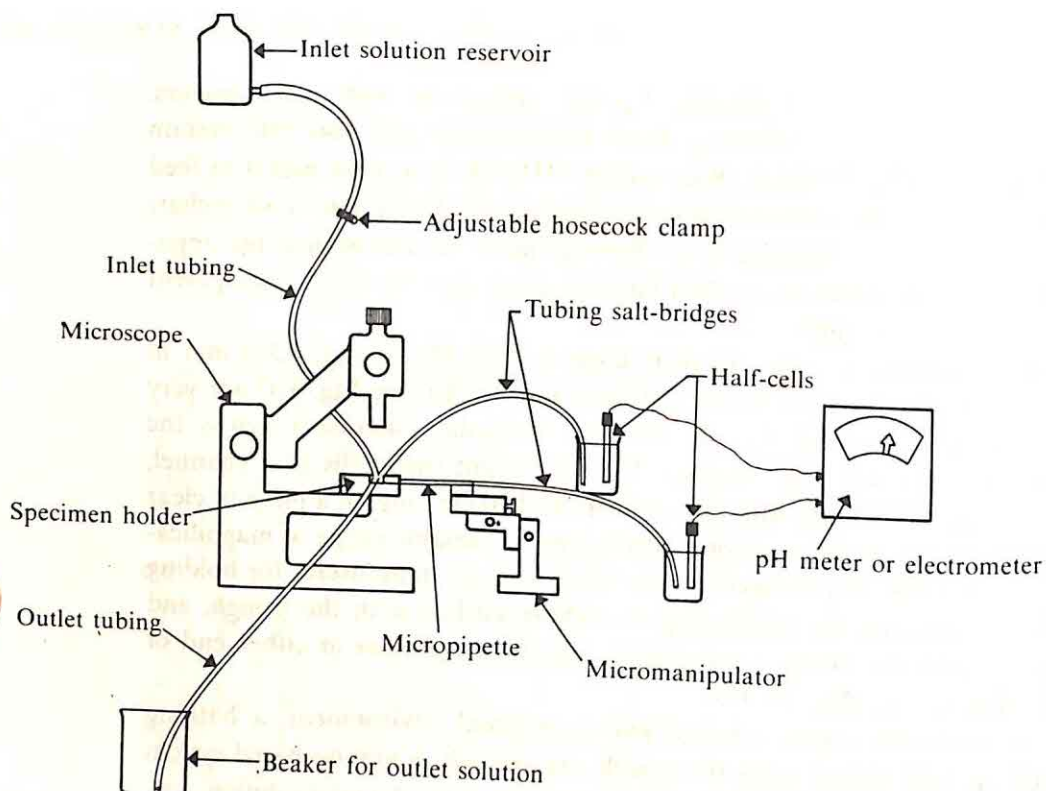
Both micropipettes and biological membranes have high electrical resistances. Moreover, we do not want to disturb the cell or change E_M by causing any appreciable current into or out of it while measuring the electrical potential differences across its membranes. Hence, the electrical potential measuring device must be capable of detecting rather small potentials (near 0.1 volt) without drawing an appreciable current, which means that here it must have an input resistance (R_{in}) of at least 10^8 ohms and preferably at least 10^{10} ohms. Since the membranes of the cell (resistance = R_{memb}), the micropipette (R_{mic}), the two tubing saltbridges (R_{sb}), the two half-cells (R_{hc}), and the electrical potential measuring device (R_{in}) are all in series, the input resistance of the latter must be large compared with the other resistances in the circuit, i.e., $R_{in} \gg R_{memb} + R_{mic} + R_{sb} + R_{hc}$. Otherwise, we could cause excessive current (i) across the membrane, and this might change its properties—from Ohm's law, $E_M = i (R_{memb} + R_{mic} + R_{sb} + R_{hc} + R_{in})$. Also, the electrical potential drop (ΔE_{in}) measured across R_{in} of the potential measuring device would not be close to E_M [$\Delta E_{in} = iR_{in} = E_M R_{in} / (R_{in} + R_{memb} + R_{mic} + R_{sb} + R_{hc})$]. Appropriate electrical

characteristics for determining E_M are obtainable with electrometers, e.g., Cary Model 31 Vibrating Reed Electrometer, and also with certain laboratory pH meters, e.g., Radiometer PHM 26. It is often useful to feed the output of the electrical potential measuring device into a strip-chart recorder. To help minimize stray electrical fields, we can enclose the apparatus in a wire shield (a so-called Faraday cage) and connect the equipment to a common ground.

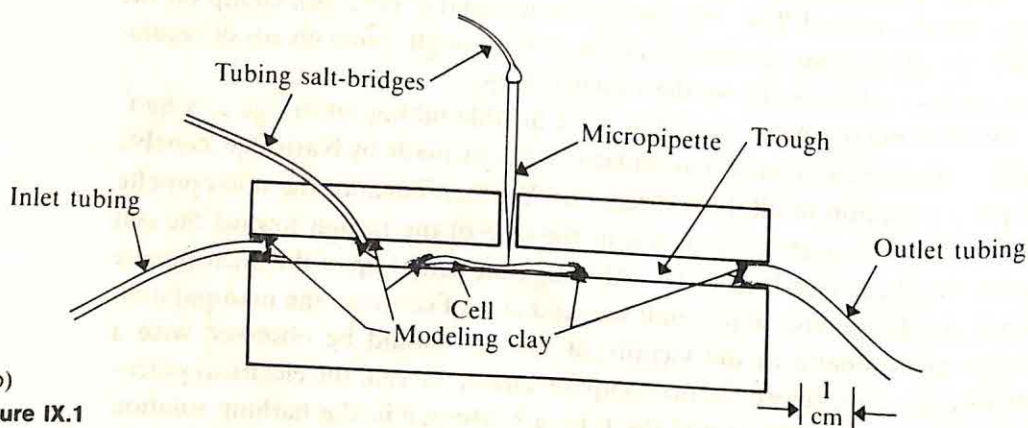
Examining cells. Due to their large size (2–10 cm long, 0.5–2 mm in diameter), the internodal cells of *Chara* and *Nitella* (see Fig. 3.4) are very convenient for measuring the electrical potential differences across the plasmalemma and the tonoplast. We can mount such cells in a channel, or trough about 5 mm wide, 5 mm deep, and 10 cm long, in a piece of clear plastic placed on the stage of a microscope—a suitable range of magnifications is 50–500. Small pieces of modeling clay are quite useful for holding the cell in place, for positioning the tubing saltbridge in the trough, and for securing the tubing for the inlet and outlet solutions at either end of the channel (see Fig. IX.1b).

To maintain a constant and known external environment, a bathing solution flows slowly along the trough past the cell. A gravity-based system works quite well, and entails placing the reservoir for the inlet solution, e.g., a 500 ml flask with a hole near the bottom for attaching tubing, above the chamber, e.g., 30–40 cm higher, while the beaker used to collect the outlet solution is placed at a level lower than the chamber (see Fig. IX.1a). To regulate the rate of flow, we can put an adjustable hosecock clamp on the tubing leading from the inlet reservoir to the trough; some means of regulation is also often useful on the outlet tubing.

A filled micropipette (attached via a flexible tubing saltbridge to a half-cell) is mounted in a micromanipulator, e.g., one made by Narishige, capable of precise motion in all three directions. We then advance the micropipette horizontally through a 2 mm slot in the side of the trough toward the cell to be “stabbed” (see Fig. IX.1b). Although the actual tip of the micropipette is not visible because of its small size and lack of contrast, the manipulation of the micropipette in the vicinity of the cell should be observed with a microscope. Just before the micropipette enters the cell, the electrical potential difference between it and the tubing saltbridge in the bathing solution should be only a few millivolts. When the micropipette pierces the relatively rigid cell wall—at which point there is an observable deformation of the cell wall—the measured electrical potential abruptly changes to a new level. Whether the new reading represents the electrical potential difference across the plasmalemma, across the plasmalemma plus the tonoplast in series, or



(a)



(b)

Figure IX.1

Arrangement of apparatus for measurement of the electrical potential differences across membranes: (a) schematic diagram indicating location of gravity-fed bathing solution, microscope, micromanipulator, and electrical circuit (b) top view of clear plastic specimen holder mounted on microscope stage. Illustration (b) shows an internodal cell of *Nitella* held in place in the trough of the specimen holder with modeling clay, which also can be used to position the tubing.

something else, depends on the intention, skill, and also the luck of the experimenter.

Experimental measurements. The composition of the solution bathing a cell affects the electrical potential differences observed across the plasmalemma, E_{pl} . The potential across the tonoplast (E_t) is generally much smaller in absolute value than E_{pl} , and unless the cytoplasmic and the vacuolar concentrations are affected, it should not be influenced by the external solution. We can learn a great deal about the relative permeability and concentration of various ions by varying the external solution and observing the resulting changes in $E_{pl} + E_t$ in series.

As we noted above, the large internodal cells of *Chara* or *Nitella* are very convenient for making electrical measurements. Upon piercing the rather rigid cell wall the micropipette often "pops" across the plasmalemma, cytoplasm, and tonoplast, directly into the large central vacuole of such cells. The reading is often steady for a matter of hours, in which time we could test the effect of a number of external solutions on $E_{pl} + E_t$. To measure the electrical potential difference across the plasmalemma, the tip of the micropipette must be in the cytoplasm. This is accomplished in a number of ways: (1) sometimes the tip of the micropipette comes to rest in the cytoplasm upon careful "stabbing" of the cell (2) if the tip is just barely protruding into the vacuole, the cytoplasm may flow along the shank of the micropipette and over the tip (3) when the tip is in the vacuole, we can advance it using the micromanipulator, so that the micropipette crosses the tonoplast and enters the cytoplasm on the far side of the cell (4) upon withdrawing the micropipette from the vacuole, the tip will at least briefly be in the cytoplasm. Micropipette tips are extremely fragile and can be broken by mistreatment or simply in passage across the cell wall.

As an experiment, we can let 1 mM NaCl plus 0.1 mM KCl be the bathing solution flowing past an internodal cell when the electrical potential differences across the plasmalemma and tonoplast are determined. Based on the measured potentials and substituting c_j for a_j in the Nernst equation (Eq. 3.5), what are the internal concentrations expected if each of the ions is in equilibrium? Are any of the calculated cytoplasmic or vacuolar concentrations far from realistic expectations? In other words, are any of the ions obviously not in equilibrium? (The cytoplasmic concentrations for *Nitella translucens* in Table 3.1 hold within a factor of 2 for most *Chara* and *Nitella* species; the vacuole may have somewhat higher concentrations of both Na^+ and Cl^- .)

Next, we could determine whether the plasmalemma is equally permeable to Na^+ and K^+ by observing the effects of these ions on E_{pl} (see pp. 114–116). For this study, we can measure the potentials for the same cell when the

- 1.6 (a) 570 sec (9.6 min)
 (b) 1.1×10^5 sec (32 hr)
 (c) Double
 (d) Increase 100-fold
- 2.1 (a) 1.5 cm
 (b) 1.25 cm
 (c) 60°
 (d) 0.75 cm
 (e) 15 m
 (f) (a) and (b)
- 2.2 (a) 22 bars, higher on 1 molal side.
 (b) 1400 bars, higher on 0.1 molal side.
 (c) 980 bars, higher on 0.1 molal side.
 (d) $\Delta P = 0$ bar, $\Delta c_{\text{solute}} = 0$ molal
 (e) Indefinite, since μ_j^* is arbitrary.
- 2.3 (a) Both 0.992
 (b) 10.7 bars
 (c) 0.74%
 (d) 0.41 mole/liter
 (e) 6×10^{-3} bar (the polymer must be influencing the osmotic pressure mainly by decreasing the activity coefficient of water).
 (f) -19.1 bars
- 2.4 (a) Both -6.00 bars (at equilibrium).
 (b) All 6.00 bars
 (c) P and $\rho_w gh$ are both 0.00 bar at the upper surface; 10 cm below the surface, $\rho_w gh$ is -0.01 bar and P is 0.01 bar; at the bottom of the tank, $\rho_w gh$ is -0.98 bar and P is 0.98 bar.
 (d) 99.6%
- 2.5 (a) $32 \mu\text{m}^3$
 (b) $12 \mu\text{m}^3$
 (c) $\frac{5}{8}$
 (d) 3.3×10^{-15} mole/chloroplast (2.0×10^9 molecules/chloroplast)
- 2.6 (a) 4×10^{-5} cm/sec (flow is inward)
 (b) None
 (c) -3.7×10^{-4} cm/sec (flow is outward)
 (d) $\cos \alpha = 0.027$ and so $\alpha = 88^\circ$
- 3.1 (a) 3.3 mM
 (b) -128 mv
 (c) 3.7×10^{-13} joule
- 3.2 (a) -128 mv (inside negative)

- (b) -120 mV
 (c) $\gamma_{\text{K-ATP}} = 0.36$; $a_{\text{ATP}} = 0.36\text{ mM}$
- 3.3** (a) K^+ is, but Mg^{++} is not.
 (b) 10 mM Na^+ inside; $0.1\text{ }\mu\text{M Ca}^{++}$ outside.
 (c) 59 mV ; 13 mM
 (d) 0.0010
 (e) μ_{Cl} is 4.1 kcal/mole higher inside the cell and μ_{Mg} is 2.7 kcal/mole lower there.
- 3.4** (a) 1 mV , negative toward the cell.
 (b) A Donnan potential of 77 mV , negative at the surface of the membrane.
 (c) -53 mV , i.e., negative inside the cell.
 (d) -55 mV ; -59 mV
- 3.5** (a) $0.5\text{ picomole/cm}^2\text{-sec}$
 (b) 0.95 kcal/mole , higher inside.
 (c) Efflux passive; influx active and requires 2.7 kcal/mole .
 (d) $0.015\text{ }\mu\text{mole/sec}$ per liter of cells
- 3.6** (a) 1.37
 (b) 4
- 3.7** (a) 50.5
 (b) 1.01
 (c) $0.91\text{ picomole/cm}^2\text{-sec}$ (facilitated diffusion requires no metabolic energy source).
- 3.8** (a) 0.78
 (b) 0.65
 (c) Yes; $-5 \times 10^{-4}\text{ cm/sec}$ (flow is outward).
 (d) $3 \times 10^{-4}\text{ cm/sec}$ (flow is inward).
- 3.9** (a) 0.75
 (b) $23\text{ }\mu\text{m}^3$
 (c) 0.00 , since the chloroplast volume did not change upon addition of glycine.
 (d) 0.5 M ; as the glycerol enters in time, the chloroplasts swell and eventually burst.
- 4.1** (a) $3.1 \times 10^5\text{ eV}$
 (b) 16°
 (c) $3.0 \times 10^{13}\text{ photons/cm}^2\text{-sec}$
 (d) Units cannot be unambiguously interconverted.
- 4.2** (a) 333 nm in a vacuum, about 0.1 nm less in air, and 222 nm in the dense flint glass.
 (b) Yes, but transition is improbable.
 (c) Yes, it has enough energy.
 (d) $30,000\text{ cm}^{-1}$

- 4.3** (a) 8.4×10^{15} photons/cm²-sec
 (b) 0.035 cal/cm²-min; 2400 μ watts/cm²
 (c) 60%
 (d) 2.9 μ m
- 4.4** (a) 2.5×10^{-9} sec
 (b) 0.75, when possible phosphorescence from the excited triplet is included.
 (c) Essentially 10^{-12} sec
- 4.5** (a) 23 μ M
 (b) 0.46; absorbance unchanged.
 (c) 3.3×10^{12} molecules/cm³-sec (5.5×10^{-9} mole/liter-sec); -1.1×10^{-4} /sec
 (d) 0.50
- 4.6** (a) 472 nm and 532 nm (distances in nm from main band are not equal).
 (b) 67%
 (c) 12 μ M
- 4.7** (a) 0.12 eV
 (b) A transition from the first excited vibrational sublevel of the ground state to the same vibrational sublevel in the excited state as for the 450 nm transition; the Boltzmann factor, $e^{-E/kT}$, is 0.010 for an E of 0.12 eV at 20°, so the transition is only 1% as probable as the 450 nm transition, cf. the indicated ϵ_{λ} s. (Also, the population of the first excited vibrational sublevel of the ground state decreases as the temperature decreases.)
 (c) From the lowest vibrational sublevel of the ground state to the third vibrational sublevel (second excited vibrational sublevel) of the excited state.
 (d) 291 nm; 0.91×10^5 liters/mole-cm (two conjugated systems of 5 bonds each).
- 5.1** (a) 0.05
 (b) 33 hr
 (c) 897.0
 (d) Maximum absorption occurs along diameter (40 μ m)— A is 0.54 in the blue region and 0.40 in the red region.
- 5.2** (a) 675 nm with 8 double bonds in conjugation; 862 nm with 10.
 (b) Only the one with 8 double bonds can.
 (c) Blue light excites the Soret band of Chl a , which rapidly becomes de-excited to the lower excited singlet (cf. Fig. 4.5), therefore the energy available is given by the red fluorescence of Chl a (cf. Fig. 5.3). The $\lambda_{\max}$ in the absorption spectrum of the pigment with 10 double bonds in conjugation is at 714 nm, thus it can become excited by resonance transfer from Chl a while the one with the $\lambda_{\max}$ at 580 nm essentially cannot.
- 5.3** (a) 0.24; 0.43
 (b) 5.8×10^{15} photons/cm²-sec; 2.4×10^{15} molecules/cm²

- (c) 2.4 times/sec; 0.17
- (d) 7.3×10^{15} molecules/cm²-min; 9.2×10^8 molecules/min per chloroplast
- (e) 0.44 times/sec; 0.91

- 5.4**
- (a) $10h\nu/O_2$
 - (b) 200 chlorophylls/photosynthetic unit
 - (c) 0.05 sec
 - (d) The uncoupling of ATP formation from electron flow is analogous to disengaging a clutch, which allows a motor to run faster; the O_2 evolution therefore initially speeds up. After a while, the lack of ATP formation causes no CO_2 to be fixed; the electron acceptors therefore stay reduced and electron flow to them is curtailed. Moreover, electron flow may eventually switch over to the pseudocyclic type, which involves no net O_2 evolution.
- 5.5**
- (a) The 710 nm light absorbed by Photosystem I leads to cyclic electron flow and accompanying ATP formation. In the idealized example given, 550 nm light by itself leads to excitation of Photosystem II only, and no photophosphorylation.
 - (b) Accessory (or auxiliary) pigments, presumably carotenoids, which are isoprenoids.
 - (c) 1.00 at 710 nm and 0.23 at 550 nm
 - (d) 7.5×10^{10} photons/cm²
- 6.1**
- (a) $\Delta G = -4.09$ kcal per mole of reactant or product, hence the reaction proceeds in the forward direction.
 - (b) $\Delta G = 0$ kcal per mole of reactant or product, which means that the reaction is at equilibrium and does not proceed in either direction.
 - (c) $\Delta G = 4.09$ kcal per mole of reactant or product, hence the reaction proceeds in the backward direction.
 - (d) 10^3 molal⁻¹
- 6.2**
- (a) 0.010
 - (b) 0.056 v
 - (c) 0.056 v, positive for the $A-A^+$ half cell; electrons flow toward the $A-A^+$ half-cell.
 - (d) $[A^+] = [B] = 1.46$ molal
 $[A] = [B^+] = 0.54$ molal
 - (e) Answer unchanged
- 6.3**
- (a) 4.2×10^{-12} M
 - (b) 1 mM ATP; 12.0 kcal/mole ATP
 - (c) 305 mv
 - (d) 14.1 kcal/2 moles of electrons; yes.
- 6.4**
- (a) 0.041 v
 - (b) 0.20 μ M

- (c) 0.43 v
 - (d) 0.24 v
 - (e) 0.28 v
- 7.1**
- (a) 0.09 cm; 0.4 sec/cm
 - (b) 0.0084; 6 μm
 - (c) 120 times higher in the pores ($A/A^{\text{st}} = 120$)
 - (d) 1.5 sec/cm
 - (e) 0.1 sec/cm
 - (f) 0.4 sec/cm
- 7.2**
- (a) 2.6 sec/cm
 - (b) 2.7 sec/cm; 2.4 sec/cm; 2.4 sec/cm
 - (c) 1.3 sec/cm
 - (d) 1.9 sec/cm
 - (e) 5.8 $\mu\text{g}/\text{cm}^2\text{-sec}$
 - (f) 0.53 $\mu\text{mole}/\text{cm}^3$
- 7.3**
- (a) 22
 - (b) 34
 - (c) 1.6 sec/cm
 - (d) 2.3 sec/cm for each
 - (e) 0.05 sec/cm; 0.2 sec/cm
 - (f) 4.2 sec/cm
- 7.4**
- (a) 3.5 $\mu\text{moles}/\text{cm}^3\text{-sec}$
 - (b) 45 μM
 - (c) 10.6 sec/cm; 0.85 $\text{n mole}/\text{cm}^2\text{-sec}$
 - (d) 8.7 sec/cm; 1.26 $\text{n moles}/\text{cm}^2\text{-sec}$
 - (e) 0.24 μM ($J_{\text{CO}_2}^{\text{r+pr}} = 0.66 \text{ n mole}/\text{cm}^2\text{-sec}$; 10 ppm corresponds to about 0.41 $\text{n mole CO}_2/\text{cm}^3$).
 - (f) 14 μM
- 7.5**
- (a) 17° for the cloudy sky, 42° for the clear one.
 - (b) 10.2 μm for the leaf; 10.0 μm for the surroundings under the cloudy sky and 9.2 μm under the clear one.
 - (c) 1.37 $\text{cal}/\text{cm}^2\text{-min}$
 - (d) 73%
 - (e) 0.22 $\text{cal}/\text{cm}^2\text{-min}$
- 7.6**
- (a) $R_{\text{wv}}^{\text{total}}$ increases from 2.8 sec/cm to 8.8 sec/cm; J_{wv} decreases 68%.
 - (b) Because of the lower J_{wv} , T^{leaf} initially increases, and more IR is therefore emitted by the leaf.
 - (c) $R_{\text{wv}}^{\text{total}}$ decreases to 2.5 sec/cm; J_{wv} increases 12%.
 - (d) Because δ^{a} is halved, J_{H}^{c} initially doubles; T^{leaf} becomes closer to T^{ta} . If T^{leaf} is above T^{ta} , as generally occurs during the day, the decrease in T^{leaf} in response to a higher wind will reduce c_{wv}^{m} (which is nearly at

the saturation value). If the accompanying fractional reduction in $c_{wv}^m - c_{wv}^{ta}$ is more than the fractional decrease in R_{wv}^{total} , the increase in wind speed will lead to a *decrease* in transpiration, since $J_{wv} = (c_{wv}^m - c_{wv}^{ta})/R_{wv}^{total}$ by Equations 7.11 and 7.12.

- 7.7** (a) 0.14 cal/cm²-min
 (b) 25 μ moles/cm²-min (0.42 μ mole/cm²-sec)
 (c) 3.0 sec/cm; 2.3 sec/cm
 (d) 12 sec/cm; 29 sec/cm
 (e) Transpiration decreases 75%, while photosynthesis decreases only 48%.
- 8.1** (a) 1.54 μ moles/cm²-sec; 0.34 μ mole/cm³
 (b) 0.22 sec/cm during peak transpiration, 0.44 sec/cm at night.
 (c) 0.07 μ mole/cm³, which corresponds to a drop in relative humidity of 7% at 20°.
 (d) -2.2 nmoles/cm²-sec; -0.48 nmole/cm³
 (e) 6800 cm²/sec
 (f) 0.4 sec by eddy diffusion, 10⁴ sec (3 hr) by ordinary diffusion.
- 8.2** (a) 7.9 at 100,000 lux (essentially none of the leaves are below light compensation), 4.6 at 10,000 lux, 1.3 at 1000 lux, and 0.0 at 0 lux (all of the leaves are then below light compensation).
 (b) 0.30 nmole/cm²-sec upward at the soil, -1.54 nmoles/cm²-sec downward into the canopy.
 (c) 390
 (d) 6.8 m above the ground (only leaves beneath this level are below the light compensation point and thus have a net evolution of CO₂).
 (e) About 12 m above the ground.
- 8.3** (a) 0.17 cal/cm²-min
 (b) 0.015 cal/min; 6×10^{-6} cal/cm²-min
 (c) -0.01 cal/cm²-min
 (d) 0.16 cal/cm²-min ($\Delta J_{wv} H_{vap} = 0.02$ cal/cm²-min)
 (e) 1.9°/cm
- 8.4** (a) 0.14 cm/sec (5 m/hr)
 (b) -0.87×10^3 dynes/cm³ or -0.87×10^{-3} bar/cm (-0.087 bar/m)
 (c) 1.1×10^{-8} cm/sec (3.9×10^{-7} m/hr)
- 8.5** (a) 8.3×10^4 sec (23 hr) for 1 cm; 8.3×10^8 sec (26 years) for 1 m
 (b) 0.017 cm/sec (0.60 m/hr)
 (c) 60 sec for 1 cm; 1.7 hr for 1 m. These are considerably shorter times than are needed for diffusion—see (a). Also, the distance traversed is proportional to time.
 (d) 0.90 m/hr for flow downward or 0.30 m/hr for movement upward, depending on the direction of the pressure gradient.

- 8.6** (a) $15\ \mu\text{m}$ for the wet soil, $0.1\ \mu\text{m}$ for the dry one.
(b) $6 \times 10^{-5}\ \text{bar}$
(c) $0.6\ \text{bar}$
(d) $-0.5\ \text{bar/m}$
(e) $-2 \times 10^{-5}\ \text{bar}$
(f) There is no net flow, since Ψ^{soil} then equals Ψ^{ta} .

(**Boldface** page numbers refer to figures or structural formulas.)

- Absorption band, 199, 203–204, 223
- Absorption coefficient, 199–201
 - foliar, 378–379
 - molar, 201
- Absorption spectrum, 193, 198, 199, 204
 - carotenoids, **226**
 - Chl *a*, 219–220, **219**
 - phycobilins, **229**
 - phytochrome, 206–207, **207**
- Absorptivity, 347
 - blackbody, 351
 - leaf, 350–351, 355
- Accessory pigments, 224–230
- Action spectrum, 193, 204–205
 - enhancement of O₂ evolution, 243–244, **245**
 - photosynthesis, 242–243, **243**
 - phytochrome, 207–209, **208**
- Active transport, 118
 - carriers, 133
 - energy required, *Nitella*, 131–132
 - Na-K pump, 131
- Activity, thermodynamic, 60
- Activity coefficient, 60, 97
 - ions, 95–97, 100
- Adhesion, 48
- ADP. *See* ATP;
 - photophosphorylation
- Advection, 372
- Arrhenius equation, 124
- Atomic orbitals, 179–180
- ATP, 272, **277**
 - bonds, 276
 - coupling sites, 284–285
 - energy currency, 260, 271, 276–277
 - formation reaction, 272–273
 - equilibrium constant, 274
 - Gibbs free energy, 275
 - magnesium, 272, 274
 - mechanisms, 286–288, 293
 - redox potential changes, 284
- Bacteriochlorophyll, 218–219, 238
- Beer's law, 200
- Bicarbonate, 330–331
- Blackbody, 170, 173–174, 295
 - absorptivity, 351
 - emissivity, 349, 351
- Boltzmann energy distribution, 121–122, 168–169
- Bouguer-Lambert law, 378
- Boyle-Van't Hoff relation, 72–75
 - irreversible thermodynamics, 153–154
- C-4 plants, 285, 336, 372
- Candle (candela), 170

- Capacitance, electrical, 94-95
 - membrane, 94
- Capillary rise, 48-51
 - contact angle, 49-50
 - xylem, 51-52
- Carbohydrate, 215-216
- Carbon dioxide
 - compensation point, 338
 - concentration
 - above canopy, 372-373
 - night, 381-382
 - plant community, 380-381
 - diffusion coefficient, 18, 327, 329
 - fixation, 214-216. *See also*
 - Photosynthesis
 - fluxes, 339-342, 373, 380-381
 - Michaelis constant, 334
 - partition coefficient, 329-330
 - permeability coefficient, 331, 332
 - resistances, 325-327, 329-333, 337-341
 - solubility, 329-330
- β -carotene, 227, 228
 - absorption spectrum, 226
- Carotenes, 227
- Carotenoids, 225-228, 245
 - absorption bands, 226-227, 226
 - photooxidations, 227-228
- Carrier, 133
- Casparian strip, 8, 390
- Cellulose, 34
 - Young's modulus, 38
- Cell wall, 1, 32-41, 33
 - components, 33
 - diffusion across, 35-37, 329-331
 - elasticity, 38, 40
 - flow in, 394
 - interstices, 34, 394
 - water vapor, 320
 - mesophyll area, 327
 - pits, 36
 - resistance, CO_2 , 329-331
 - stress-strain relations, 38-41, 39
 - water, 34, 329
 - hydrostatic pressure, 83-85
 - Young's modulus, 38, 40
- Chemical potential, 54-63, 105-106, 262, 455-456
 - active transport, 118
 - protons, 286-287
 - See also individual terms*
- Chemi-osmotic hypothesis, 286-288, 293
- Chl *a*, 217, 218
 - absorption spectrum, 219-220, 219
 - in vivo*, 222-223
 - fluorescence, 219, 221-222, 237-238
 - depolarization, 224
 - resonance transfer, 233-234, 236
 - vibrational sublevels, 220-222, 222
- Chl a_{670} , 222, 236, 244-245
- Chl a_{680} , 222, 244
- Chl *b*, 217-218, 223, 244
- Chl *c*, 218, 236
- Chlorophyll
 - concentration *in vivo*, 201, 341
 - de-excitations, 186-189
 - fluorescence, 186, 192
 - leaves, 201, 341
 - light absorption, 183-185
 - phosphorescence, 187
 - types, 217-218
 - See also* Chl *a*; P_{700}
- Chloroplasts, 24-25, 24
 - bioenergetics, 279-288
 - electron transfer, 246-256
 - frequency per cell, 4
 - lamellar membranes, 25
 - light absorption, 201
 - osmotic responses, 75-77, 154
 - permeability, 154-156, 333
 - photosynthesis, 333-335
 - reflection coefficients, 154-156
 - resistance, CO_2 , 333
 - stroma, 25
 - volume, 71, 76
- Chromoplasts, 225
- Clay, 382, 387, 393
- Cloudlight, 345-346
- Cohesion, 48
- Compensation point,
 - CO_2 , 338
 - light, 339, 379-380
- Conjugation, 201-204
 - absorption band, 203-204
- Constant field equation, 113-114
- Contact angle, 49-50
 - cell wall, 85
- Continuity equation, 12
- Coulomb's law, 53
- Coupling sites. *See* Oxidative

- phosphorylation;
 - Photophosphorylation
- Conduction (heat), 356-358
- Convection (heat), 356
- Crassulacean acid metabolism, 342
- Cuticle, 3
 - resistance, 310
 - transpiration, 306-307
- Cutin, 3
- Cyclic electron flow, 255, 284
- Cytochromes, 251-254
 - chloroplasts, 250-252
 - mitochondria, 290-291
- Darcy's law, 386-387
- Debye-Hückel equation, 96
- De-excitations, 185-189
- Dew formation, 359-361
- Dielectric constant, 53
- Diffusion, 9
 - time-distance relationship, 14-15
 - See also* Fick's laws
- Diffusion coefficient, 11, 105
 - eddy, 369-370, 375
 - gases, 18, 327
 - in membranes, 22-23
 - small solutes, 17
- Diffusion potential, 106
 - membrane, 111-114
 - micropipettes, 108
 - solution, 106-108
- Diurnal changes
 - soil-plant-atmosphere, 406-408
 - tree diameter, 404
- Donnan potential, 116-118
- Eddy diffusion coefficient, 369-370
 - above canopy, 370
 - plant community, 375
- Electric dipole, 176
- Electrical potential, 92
 - capacitor, 94-95
 - membrane, 99, 109
 - Nitella*, 114-116
- Nernst, 97-99
- Electrical term, chemical potential, 61, 92
- Electrodes. *See* Half-cells;
 - Micropipettes
- Electrogenicity, 119-121
- Electron
 - charge, 93
 - energy, 268-270
 - orbitals, 177-178
 - role in light absorption, 175-176
 - spin, 177-178
 - transitions, 184-185
- Electron transfer
 - ATP formation, 284-285
 - chloroplasts, 246-256, 280-283
 - types, 254-255, 284
 - mitochondria, 289-291
- Electroneutrality, 93, 95, 112
- Emissivity, 295
 - blackbody, 349, 351
 - leaf, 351-352
- Energy balance (leaf), 343-345
 - storage terms, 344
- Energy currencies, 260, 271
- Energy flow, biosphere, 293-298
- Energy level diagram, 192-194
 - chlorophyll, 183
- Energy radiated, 294-295
 - solar, 295
 - Stefan-Boltzmann law, 295
- Enhancement effect, Emerson, 243-244
- Entropy, 10, 451-453
- Epidermis
 - hairs, 318
 - leaf, 3
 - root, 8
- Equilibrium, 56, 100, 102
 - entropy, 451-452
 - Gibbs free energy, 261, 453
 - water, 71
- Equilibrium constant, 265
 - ATP, 274
 - biochemical conventions, 273-274
- Exchange diffusion, 137
- Excitation transfer, 189, 224-225, 231
 - efficiency, 235
 - resonance, 232-234
 - trapping, 237-238
 - wavelengths, 235-236
- Evapotranspiration, 373
- Facilitated diffusion, 136-137
- FAD-FADH₂, 277, 289-290

- Faraday, 93
 Ferredoxin, 250, 254, 283-284
 Fick's first law, 10-11, 105, 322
 cylindrical geometry, 389
 resistances, 304, 311-313
 Fick's second law, 13
 Flavoprotein, 290
 Fluorescence, 186, 197
 chlorophyll, 186, 192
 delayed, 187
 energy, 197
 lifetime, 186, 188
 Fluxes
 cylindrical geometry, 388-389
 irreversible thermodynamics,
 139-141
 solute, 112
 irreversible thermodynamics,
 464-465
 volume, 142-143
 water, 85-86
 Flux ratio equation, 124-126
 Foliar absorption coefficient, 378-379
 Food chain, 297-298
 Force-flux relationship, 11, 57,
 101-102, 303
 irreversible thermodynamics,
 138-139
 Franck-Condon principle, 196-198
 Free energy, 55. *See also* Chemical
 potential; Gibbs free energy
 Frost formation, 359-361
 Fucoxanthin, 227, 235, 244

 Gibbs free energy, 56, 261-263,
 453-455
 ATP formation, 275-276
 chemical reaction, 263-266
 enrichment per atom, 294
 equilibrium, 261-262, 453
 equilibrium constant, 265
 glucose to ATP, 294
 light absorption, 282
 photosynthesis, 215-216
 Global radiation, 347
 Goldman equation, 113-114
 Nitella, 114-116
 Graham's law, 18
 Gravitational term, chemical
 potential, 62

 Grotthus-Draper law, 175
 Guard cells, 4, 307

 Half-cells, 266-269, 468
 hydrogen, 270
 water-oxygen, 280
 See also Redox potentials
 Heat fluxes, 343-345
 conduction, 356-358
 convection, 356
 dew or frost formation, 359-360
 transpiration, 358-359
 Heme, 252, 252
 Henry's law, 459
 Hill reaction, 247
 Hund's rule, 178
 Hydraulic conductivity coefficient,
 143, 463
 soil, 386-388
 Hydrogen bond, 45-47, 46
 Hydrogen half-cell, 270

 Ice, heat of fusion, 46
 Ideal solution, 60
 Incipient plasmolysis, 78-80
 reflection coefficients, 150-153
 Infrared, 165
 absorption, leaf, 347-349
 emission
 leaf, 349
 sky, 348
 Intercellular air spaces, 4
 resistance, 310-311
 Ionic strength, 97
 Irreversible thermodynamics,
 138-140, 461-465
 conjugate forces and fluxes,
 461-463
 diffusional flux, 461, 462-463
 dissipation function, 462
 water and solute flow, 140-143
 Isoprene, 225

 Lamps, 170-171, 173-174
 Leaf
 cells, 3-4, 3
 energy balance, 343-345
 energy storage, 344

- freezing, 355-356
- IR absorption and emission, 347-352
- palisade mesophyll, 4
- radiation balance, 352-353
- shade or sun, 328, 341
- shaded, 361-362
- silvery, 355
- spongy mesophyll, 4
- steaming, 374
- unstirred air layer, 303-306
- water potential, 83, 407-408
- wilting, 402-403, 407-408
- See also* Resistances
- Leaf area index, 378
- Lifetime, 186, 190-191, 450
- Light
 - absorption, 175-176
 - chlorophyll, 183-185
 - probability, 176, 193
 - redox effects, 280
 - time, 185-186
 - compensation point, 339, 379-380
 - energy, 166-169
 - units, 170-171
 - velocity, 165-166
 - wave number, 210
 - wavelength, 164-165
- Lignin, 34-35
- Lutein, 227, 228
 - absorption spectrum, 226
- Matric potential, 67-69, 385-386
- Membranes
 - capacitance, 94
 - composition, 19-21
 - models, 19, 20, 21-22
 - permeability, 26
 - potentials, 111-114
 - Nitella*, 114-116
 - Q_{10} for diffusion, 121-123
 - resistance, 120
 - water flow, 395
- Mesophyll cells, leaf, 3-4
 - area, 327-329, 341
 - resistance, CO_2 , 332
- Michaelis-Menten formalism, 134-136
- Microbodies, 25
- Microfibril, cell wall, 34
- Micropipettes, 108, 466-467
 - diffusion potentials, 108
- Mitochondria, 23-24, 23
 - bioenergetics, 288-293
 - electron transfer components, 289-291
 - oxidative phosphorylation, 291-293
- Mobility, 103, 108
- Mole fraction, 65
- Molecular orbitals, 179-181
 - antibonding or bonding, 181-182
- NADP-NADPH₂, 254, 277-279, 277
 - energy currency, 260, 271, 283
 - redox couple, 277-279
- Nernst potential, 97-99
 - potassium, *Chara*, 99-100
- Noncyclic electron flow, 254, 284
- Ohm's law, 102, 337
- Onsager coefficients, 140, 463
- Osmotic coefficient, 73, 154
- Osmotic pressure, 64-67
 - chloroplasts, 75-77
 - incipient plasmolysis, 78-80, 150-153
 - reflection coefficients, 151-152
 - wilting, 403
- Oxidation-reduction potentials. *See* Redox potentials
- Oxidative phosphorylation, 291-293
- Ozone, 172-173
- P_{700} , 222-223
 - excitation trapping, 237-238
 - photochemical reaction, 189
 - Photosystem I, 244, 254
 - redox potential, 283
- Palisade mesophyll, 4, 327-328
- Partition coefficient, 28, 63
 - CO_2 , 329-330
- Pauli exclusion principle, 177-178
- Pectin, 35
- Permeability coefficient, 29
 - cell wall, 36
 - CO_2 , 331, 332
 - Nitella*, 114-115
 - series barriers, 42

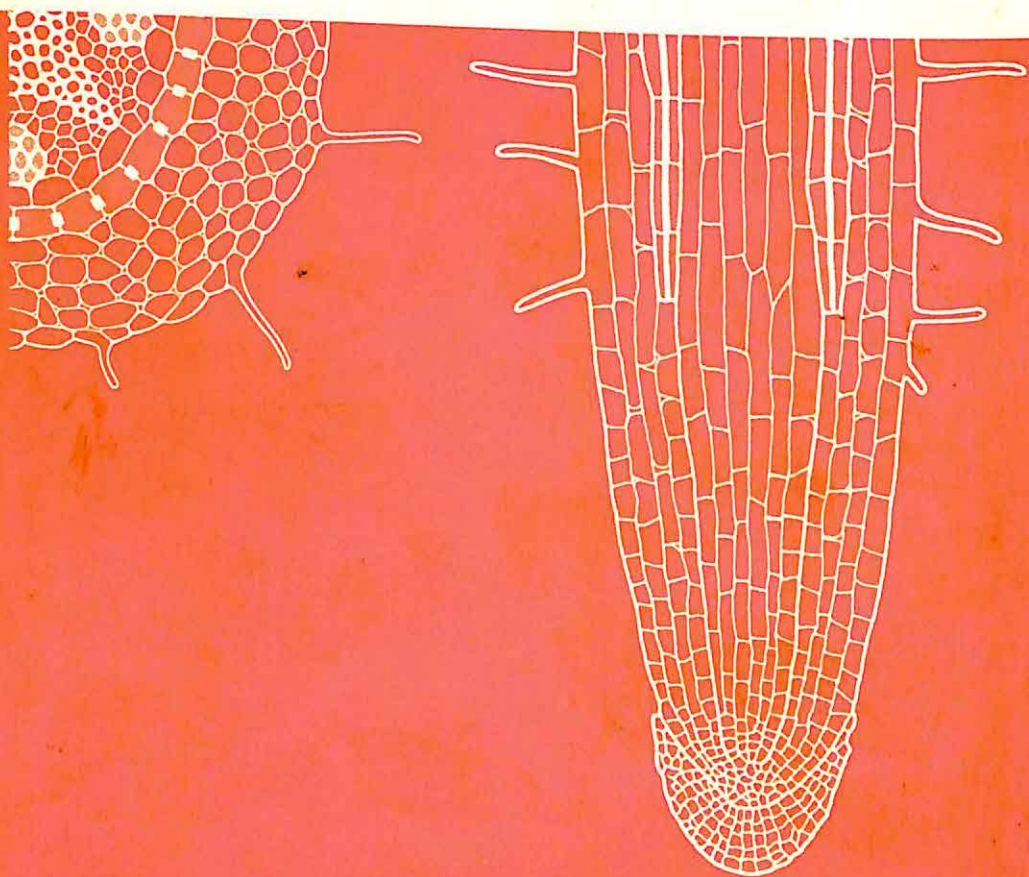
- Permeability coefficient (continued)
 small solutes, 32
 water, 32, 87, 332
- Peroxisomes, 1, 25, 335
- Phenomenological equation, 140
- Phloem, 4, 5, 6, 395-396
 contents, 396-397
 mechanism of flow, 397-401
 sieve plates, 396, 398
 sieve tubes, 396
 solute velocity, 397
 water potential, 397-401
- Phospholipid, 20, 20
- Phosphorescence, 187
- Photochemistry
 laws, 175
 reaction, 189, 231
 time, 247
- Photoelectric effect, 163, 168
- Photoisomerization, 182
- Photon, 166
- Photophosphorylation, 247, 256
 coupling site, 284-285
 Gibbs free energy, 275-276
 mechanisms, 286-288
 redox potential, 284
- Photorespiration, 335-336, 338
- Photostationary state, 209-210
- Photosynthesis, 214-216
 action spectrum, 242-243, 243
 chemical reaction, 214-215
 dark reactions, 214, 283-284
 efficiency, 216, 284, 296-297
 electron flow, 246-256
 enhancement, 243-244
 Gibbs free energy, 215-216
 leaf, 333-335
 Michaelis-Menten formalism, 334
 net, 339-341
 oxygen evolution, 247-249
 photochemistry, 214-215, 238, 247
 photophosphorylation, 247, 256
 primary events, 214, 232
 quantum requirements, 216, 248
 times, 247
- Photosynthetic units, 239-246, 239
 excitation processing, 241-242
- Photosystem I, 244-246, 254
 redox reactions, 283
- Photosystem II, 244-246, 249
 redox reactions, 280-282
- Phycobilins, 228-230
- Phycocyanin, 228, 230, 235, 236
 absorption spectrum, 229
 enhancement of O_2 evolution, 245
- Phycocerythrin, 228, 229-230, 235, 236
 absorption spectrum, 229
 enhancement of O_2 evolution, 245
- Phytochrome, 205-210, 206
 absorption spectrum, 206-207, 207
 action spectrum, 207-209, 208
- Planck's radiation distribution
 formula, 174, 294-295
- Plasmalemma, 1
 permeability, 19, 331
 resistance, 331
- Plasmodesmata, 37
- Plasmolysis, 78
- Plastocyanin, 251
- Plastoquinone, 251, 282, 284
- Poiseuille's law, 391-392
- Porphyrin, 217
- Pressure term, chemical potential,
 60-61, 63, 456-459
- Protoplast, 1
- Pseudocyclic electron flow, 254, 284
- Pyrrole, 205
- Q_{10} , 121-123
- Quantasome, 225
- Quantum, 166
- Quantum efficiency (yield), 191-192
- Quencher, 191
- Quinone, 251, 251
- Radiation
 balance, net, 352
 leaf, 352-353
 global, 347
 solar, 295, 345-347, 350
- Radiationless transitions, 186, 188
- Raoult's law, 80, 459
- Rate constant, 188, 190-191
- Rayleigh scattering, 346
- Reaction
 first-order, 186, 190
 photochemical, 189
- Reaction center, 239, 246
- Reciprocity relation, Onsager, 140,
 463

- Redox potentials, 269-271
 - ATP formation, 284
 - chloroplast components, 250, 280-283
 - ferredoxin, 283
 - light absorption, 280
 - midpoint, 270
 - NADP-NADPH₂, 279
 - P₇₀₀, 283
 - water-oxygen, 280
- Reflection coefficient, 144, 151, 463-464
 - average, 150
 - chloroplast, 154-156
 - osmotic pressure, 150-152
 - partition coefficient, 147
 - permeability coefficient, 147-148
 - range, 145-147, 464
- Relative humidity, 80-81, 320
- Resistances, 303
 - above canopy, 371
 - cell wall, 329-331
 - chloroplast, 333
 - CO₂, 325-327, 336-338
 - cuticular, 310
 - cytoplasmic, 331-332
 - Fick's first law, 304, 311-313
 - intercellular air spaces, 310-311
 - leaf, 315
 - mesophyll, 332
 - parallel, 315
 - photosynthesis, 338, 340-341
 - plant community, 375-376
 - plasmalemma, 331
 - series, 315
 - soil-plant-atmosphere, 404-406
 - stomata, 308-310
 - transpiration, 314-318
 - unstirred air layer, 303-305, 306
 - water vapor, 314-318
- Resistivity, 102, 303
- Resonance transfer, 232-234
- Respiration, 288-291, 335
 - plant, 381-382
- Reynolds number, 392, 394
- Root
 - area, 405-406
 - casparian strip, 8, 390
 - cells, 6-8, 7, 390-391
 - water traversal, 390-391
 - water uptake, 389
- Sand, 382, 387
- Seed germination, action spectrum, 207-209, 208
- Similarity principle, 370-371
- Singlet, 177
- Sky
 - effective temperature, 348
 - light, 346-347, 350
- Soil, 382-383
 - energy balance, 384
 - hydraulic conductivity, 386-388
 - thermal properties, 383-384
 - water potential, 383, 384-386, 406-408
 - wilting, 402-403
- Solar constant, 171
- Soret band, 219
- Spin, 176-178
- Spongy mesophyll, 4
- Standard state, 62
- Stark-Einstein law, 175
- Stationary state, 148
- Steady state, 102, 107
- Stefan-Boltzmann law, 295, 348
- Stomates, 4, 306-308, 317-318
 - frequency, 308, 316
 - closure, illumination, 380
 - resistance, 308-310
 - transpiration, 323-325
- Sun
 - chlorophyll excitation, 241
 - energy radiated, 295, 345-347, 350
 - light, 171-173, 172
- Surface tension, 47-48
- Symplasm, 37, 390
- Temperature
 - coupling, leaf and air, 357-358
 - dew point, 360
 - radiation, 295, 348-349
 - sky, 348
- Temperature coefficient, 121-123
- Tensile strength
 - membranes, 71
 - water, 52-53
- Tetrapyrrole, 205, 217
- Thermal conductivity coefficient
 - air, 356-357
 - soil, 383
- Tonoplast, 1, 71

- Transpiration, 322-323
 cuticular, 307, 322
 heat flux, 358-359
See also Water; Water vapor
- Trap chl, 230-231, 237-239, 246. *See also* P_{700}
- Tree
 diffuse-porous or ring-porous, 393-394
 diurnal diameter fluctuations, 404
 Triple, 177
- Ultraviolet, 165
- Uncoupler, phosphorylation, 257
- Unstirred layers
 air, 303
 leaf, 303-305
 thickness, 305-307
 solution, 27-28
- Ussing-Teorell equation, 124-127
Nitella, 129-130
- Vacuole, central, 1-2, 71-72
- Van't Hoff relation, 65-67
- Vibrational sublevels, 192-198
- Viscoelastic, 40-41
- Volume
 nonosmotic (nonwater), 73, 74
 partial molal, 61
 water, 61, 63
- Volume flow, 142-143
 water, 86
- Water, 44-45
 activity, 64
 conductivity coefficient, 86-87
 dielectric constant, 53-54
 flux, 86, 141
 soil-plant-atmosphere, 404-405
 xylem, 394-395
 heat of vaporization, 47
 light absorption, 172-173
 partial molal volume, 61, 63
 permeability coefficient, 32, 87
 solvent, 54
 surface tension, 47-48
 tensile strength, 52-53
 velocity, 86
- Water-oxygen half-cell, 280
- Water potential, 69-70
 air, 81-83
 leaf, 83, 407-408
 phloem, 398-401
 soil, 383, 384-386, 402, 407-408
 wilting, 402-403, 407-408
- Water vapor
 activity, 80
 concentration
 above canopy, 374
 leaf, 318-322
 plant community, 376-377
 diffusion coefficient, 18
 fluxes
 relative to CO_2 , 342
 leaf, 322-323
 partial pressure, 80-81
 resistances, 314-318
 leaf, 315
 unstirred air layer, 306
 saturation pressure, 80
- Wave number, 210, 349
- Wien's displacement law, 174, 348
- Wilting, 325
 soil water potential, 402-403, 407-408
- Wind velocity, 306, 367-368
 above canopy, 367-368
 plant community, 375
 unstirred layers, 305, 367-368
- Xanthophylls, 227
- Xerophytes, 83, 328, 403
- Xylem, 4-6, 5, 391
 capillary rise, 51-52
 diffuse-porous or ring-porous, 393-394
 vessel, 391
 water flow, 394-395
- Young's equation, 49
- Young's modulus, 38



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